

**AUTOTOMY IN TUBULARIA CROCEA
AND ITS ECOLOGICAL
AND PHYSIOLOGICAL SIGNIFICANCE**

INAUGURAL DISSERTATION

**ZUR ERLANGUNG DER PHILOSOPHISCHEN DOKTORWUERDE VORGELEGT
DER PHILOSOPHISCHEN FAKULTAET II DER UNIVERSITAET ZUERICH**

VON

DURI RUNGGER

AUS VERSAM / GR

1969
—

BEGUTACHTET VON PROF. DR. P. TARDENT

ZUERICH 1968

AUTOTOMY IN TUBULARIA CROCEA
AND ITS ECOLOGICAL
AND PHYSIOLOGICAL SIGNIFICANCE

INAUGURAL DISSERTATION

ZUR ERLANGUNG DER PHILOSOPHISCHEN DOKTORWUERDE VORGELEGT
DER PHILOSOPHISCHEN FAKULTAET II DER UNIVERSITAET ZUERICH

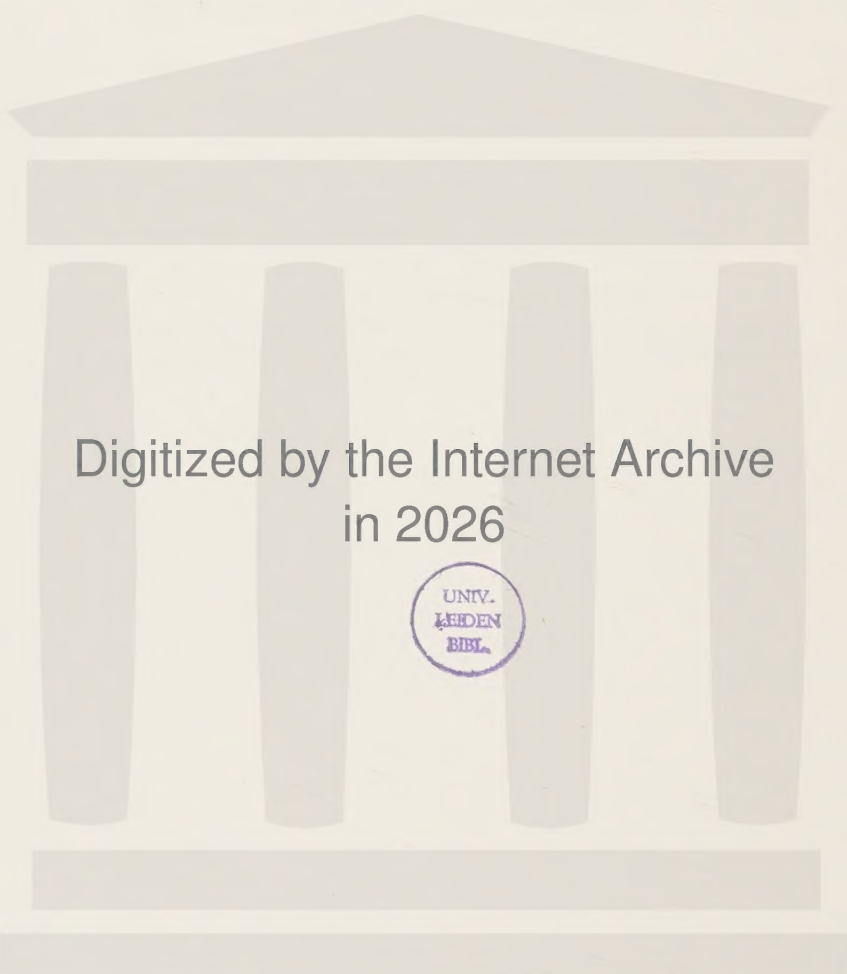
VON

DURI RUNGGER

AUS VERSAM / GR

BEGUTACHTET VON PROF. DR. P. TARDENT

ZUERICH 1968



Digitized by the Internet Archive
in 2026



Autotomy in *Tubularia crocea* and its ecological and physiological significance

by

DURI RUNGGER

(From the Zoological Institute, University of Zurich and the Zoological Station of Naples)

15 Figures

(Received January 6, 1969)

Summary. The present investigation is concerned with the phenomenon of hydranth autotomy in *Tubularia crocea* AGASSIZ and the ecological and physiological aspects related to it.

1. The process of hydranth autotomy is described in detail.

2. Colonies of *Tubularia* from the Naples area possess mature hydranths and show autotomy all the year round. As to the autotomy-regeneration cycle, there are no seasonal fluctuations nor true regression periods. Among natural populations the frequency of autotomy varies from colony to colony.

3. Colonies under identical water conditions show a marked correlation with respect to the autotomy-regeneration cycle but the extent of autotomy still varies from colony to colony. Ecological stresses as represented by a raising of the water temperature and PCO_2 favour autotomy.

4. It has never been observed that sessile or single hydranths produce 2 consecutive gonophore generations. Regenerates replacing autotomized hydranths reach maturity.

5. Spontaneously autotomized and amputated hydranths continue to live separated from the colony for as long as 30 days. During this time they feed, continue to mature and to spawn sexual products (actinulae, spermatozoa) and are capable of regenerating lost parts. Isolated hydranths show cell proliferation as mature hydranths attached to the stem.

6. The longitudinal growth of the caulus is related to the autotomy-regeneration cycle. During the 2 days preceding autotomy growth is slowed down or stops completely in both single polyps and colonial specimens. This phenomenon is accompanied by a temporary decrease in the rate of cell divisions.

In small colonies repression of growth is correlated in all individuals. When one polyp enters autotomy growth of all other members of the colony is inhibited.

7. Two hydranths grafted together with opposite polarity exhibit an increased tendency for autotomy compared with single polyps. Individuals entering autotomy and showing advanced incision reconstitute the incised neck region when the caulus is cut near the incision.

8. Diffusion extracts obtained from minced colonies greatly increase the rate of autotomy when added to the culture water.

9. The significance of the findings summarized in 1-8 are discussed:

The autotomy-regeneration process expressed by a repeated renewal of the hydranth appears to be an integrated part of the life cycle of *Tubularia*. As liberated hydranths continue to give off spermatozoa and actinulae they may well, when carried away by water movements, contribute to the horizontal propagation of the species, thus replacing the missing medusa stage. Accumulation of unfertile members in the colonies is avoided by replacement of hydranths that have passed the sexual phase and are not able to reconstitute a fertile gonophore generation.

It is suggested that spontaneous autotomy is released and controlled by a hydranth-produced inhibitor which accumulates within the colony and which also affects cell divisions as well as growth in all members of the colony.

INTRODUCTION

Regression-regeneration cycles are known from different hydroids, such as *Obelia* (BERILL, 1949; CROWELL, 1953), *Pennaria cavolini* (TARDENT, 1963, 1965), *Campanularia flexuosa* (CROWELL, 1953; NATHANSON, 1955; STREHLER, 1961) and others. In these species regression consists of a resorption of the hydranth structures. Autotomizing polyps of *Tubularia* shed instead their terminal hydranths (MORSE, 1909; MOORE, 1939; BERILL, 1948; PYEFINCH and DOWNING, 1949; TARDENT, 1952-1965). It is not yet clearly understood whether in this particular case this phenomenon serves a physiological and/or ecological purpose.

Different opinions concerning this problem have been expressed. MACKIE (1966) studied the behaviour of growing colonies of *Tubularia crocea* AGASSIZ in Villefranche. According to his observations « healthy », feeding animals kept under favourable environmental conditions do not exhibit autotomy, and will presumably live indefinitely, growing more or less steadily. TARDENT (1962, 1963, 1965) based his experiments on *Tubularia larynx* (Naples) and considered spontaneous autotomy as being the consequence of an aging process to which the naked hydranth is subjected. Senescence may in this case be the result of a gradual exhaustion of particular cell types within the hydranth (nematocysts, gland cells and gonocytes). According to his view, this depletion cannot be compensated by cell proliferation within the hydranth and/or by a supply of new cells from other parts of the polyp. As the cells of a regenerating hydranth originate from hydrocaulus tissues the periodic reconstitution of the hydranth is, according to this view, identical with complete rejuvenation of this part of the polyp. Furthermore TARDENT (1965) believes in a close relationship between the autotomy-regeneration phenomenon and subterminal growth of the polyp, cauline growth being possible only in a relatively short period after the reconstitution of a new hydranth has taken place. On the other hand mature hydranths liberated through autotomy could, at least partly, compensate for the missing medusa generation, which in *Tubularia* is reduced to sessile gonophores. In the light of TARDENT's interpretation the phenomenon assumes various physiological and ecological significance.

The purpose of the present investigation was to reexamine these hypotheses and views about the significance of autotomy in *Tubularia* by studying the behaviour of the colonies and their components in their natural environment and under artificial conditions, and by investigating the morphogenetic factors to which this phenomenon is subjected.

MATERIAL AND METHODS

In the bay of Naples the genus *Tubularia* is represented by one species only, *Tubularia crocea* AGASSIZ (VOSS-BRINCKMANN, personal communication). TARDENT (1952-1965) and other authors named it erroneously *Tubularia larynx* ELLIS and SOLANDER.

The local distribution of *Tubularia crocea* is restricted to the areas of the commercial harbour of Naples, the small harbours of Santa Lucia and occasionally Mergellina (for details about habitat see p. 98). All material used for laboratory observations and experiments was collected from floating barrels at Santa Lucia used for rearing *Mytilus*. The observations about the behaviour of free-living colonies by means of SCUBA-diving were made in the same locality. For the control of larval settlement and colony-growth at different depths untreated wooden plates (2×15 cm) were fixed to a metal cable hanging from a barrel. The distance between each plate was 1.2 m. They were examined each week.

In the laboratory, single cauli with or without hydranths and entire colonies were kept in standard dishes on a rocking table or in non-agitated sea water at 13°, 16°, 18° and 21°C. The off-shore sea water, collected in the Gulf of Naples from depths between 10-20 m, was supplied daily. Isolated hydranths were kept floating in aerated off-shore water. All animals were fed, as far as possible, daily with nauplii of *Artemia salina*.

For the autoradiographic examination of mitotic activities and cell migration 3H-thymidine (New-England Nuclear Corp. 6.7 Ci/mM) was injected into the caulus and/or hypostome 24 hours before fixing the objects in Bouin. The undiluted 3H-thymidine was injected by means of a micro-pipette controlled by a micro-syringe according to the method described by CAMPBELL (1965). The injected quantity varied with the size of the hydranth and the length of the caulus. In order to examine how far an exchange of migrating cells occurs between different regions of the polyp, fragments of animals which had been labelled 24 hours before were rinsed with sea water and grafted to non-labelled fragments. A description of the grafting procedure is given by TARDENT (1956). The longitudinal sections (7μ) were covered with Eastman Kodak nuclear bulk emulsion NTB 3. After an incubation period of 20 days the slides were developed in Kodak D19b and stained with haemalum (MAYER). The procedure by which the autoradiographs were examined is described on p. 129. For conventional histology the objects were also fixed in Bouin and the 10μ thick sections stained with haemalum.

The experiments concerned with the influence of oxygen and carbon dioxide on autotomy were set up according to the technique described by BRAVERMANN (1962). The animals were kept at high partial pressures of O_2 , CO_2 respectively at 15°C in airtight plastic boxes. In each box there were 4 standard-dishes, 2 containing the animals and 2 with sea water only. In this way the sea water was conditioned for use the next day. A stopcock at each end of the box allowed the exchange of gases. In one box the air was replaced by pure O_2 and, during the experiment, the produced CO_2 was absorbed by pumping the gas through concentrated KOH. In the other box 2 volume-percent of the air were replaced by CO_2 . After a saturation time of 24 hours during which the gas was pumped through the water, the animals were brought into the containers, fed daily and transferred to the replacement dishes. The boxes were opened for counting and feeding once a day.

The preparation of «diffusion waters» from minced colonies and of various extracts from homogenates is described with each experiment (see p. 124 ff). In order to obtain an isotonic sea water solution of 37 ‰, extracts made with distilled water were mixed with 9 parts of «instant ocean» (Aquarium system, Inc., Wickliffe, OHIO) the salinity of which read 41 ‰.

ECOLOGY OF *Tubularia crocea*1.1. *Biological data*

VOSS-BRINCKMANN (in preparation) gives a summarized description of the biology of *Tubularia crocea*. PYEFINCH and DOWNING (1949) comment on various aspects of the biology of *Tubularia larynx*, they describe the spawning and settlement of actinulae and also provide some information about occurrence and frequency of autotomy. HAWES (1958) deals with the settlement of larvae and LOWE (1926) with the development of the gonophores in the same species. Further descriptions of the gonophore development are given by BRAUER (1891) for *T. mesembryanthenum* and by AVSET (1967/68) for *T. indivisa*.

This chapter will give personal observations on *T. crocea* from Naples which are directly and indirectly related to the autotomy-regeneration phenomena.

1.1.1. *Habitat*

In Naples the colonies of *T. crocea* are found attached to large numbers of partially submerged barrels used for cultures of *Mytilus*. The colonies also grow on the old barges and floats in the commercial harbour of Naples, near Santa Lucia and occasionally also in Mergellina. Extensive inspections of other possible habitats between 0 and 20 m depth by aqua-lung diving (Santa Lucia, Riviera di Chiaia, Gaiola, Capo Posillipo, Nisida, Procida) have led to the conclusion that *T. crocea* is not to be found on rocky and sandy bottoms nor on *Posidonia* weeds or algae. SCHOENENBERGER (personal communication) has found a small colony in the bottom material collected from 6-10 m depth in front of via Caracciolo, where the bottom is muddy. BRAENDLE (personal communication) observed a single polyp attached to a *Nassa*-shell occupied by a *Pagurus* from the same place.

The vertical distribution is limited by the surface and the thermocline which, in the above described areas, oscillates between 20 and 10 m depth.

In order to study the development of colonies under natural environmental conditions at different depths, female colonies of known size were fixed to wooden plates (2 × 15 cm) and lowered to various depths along a metal cable (0-18 m depth). Unfortunately the colonies were lost because of water movements but some actinulae produced meanwhile settled on the plates. Neither in the number of cauli, nor in producing mature hydranths, nor in the occurrence of autotomy did the growing colonies show obvious differences related to depth. After 2 months 40 to 200 cauli were found on each plate down to 16.2 m depth, the actual thermocline.

The size of the colonies is variable. Some colonies found in the sea are

composed of 500 and more individuals. A close examination shows, however, that these large tufts are not true colonies but associations of different colonies grown together from different actinulae or from actinulae settled on the cauli of already established bunches. There is, however, no anastomosis between the stolons. One real colony may show up to 200 stems. As observed by SCUBA-diving the density of tufts decreases with increasing depth.

1.1.2. *Gonophore maturation*

T. crocea is strictly gonochoristic. There are female and male colonies, but due to the above-mentioned associations both female and male polyps may be found within a tuft.

According to PYEFINCH and DOWNING (1949), the first hydranths of a newly-formed colony of *T. larynx* reach maturity within approximately 24 days after settlement of the larva. Later these primary hydranths autotomize and are replaced, but the authors could not observe whether in the successive generation of hydranths the gonophores reach maturity.

My observations on settling and developing larvae in the sea (wooden plates) agree with these findings. The gonophores of newly-founded colonies of *T. crocea* reach maturity 20-30 days after settlement of the larvae at a sea temperature of around 25°C. After autotomy or amputation, regenerated hydranths mature within 8 to 14 days after reconstitution, as observed under laboratory conditions (15° and 18°C). Sometimes, however, these regenerated hydranths, as well as the primary ones, autotomize before they reach sexual maturity.

Sexual maturity is defined as follows: The gonophores of female polyps contain visible eggs or developing larvae. The appearance of ripe spermatozoa within male gonophores is indicated by their opaque, milky aspect. These situations correspond to the stages 5 and 6 of the gonophore normogenesis given in Fig. 1. The latter is based on histological studies in accordance with those given by AVSET (1967/68) for *T. indivisa*.

In the female gonophores the oogonia situated around the spadix fuse to oocytes. The degenerated nuclei of the oogonia (pseudo-cells) are, at a later stage of the development, found in the endodermis of the larvae.

The male gonophores contain all stages of spermatogenesis, spermatocytes, spermatids and ripe sperms. The milky aspect of the male gonophores increases with the amount of ripe sperms stored in the subumbrellar cavity.

Once the gonophores have liberated all spermatozoa and larvae, they detach from the blastostyles. It was never observed that a second gonophore generation was formed on the same hydranth. On the other hand, as we have seen, after autotomy and amputation, succeeding regenerates are able to ma-

ture, thus replacing successfully a hydranth that already had passed its sexual phase.

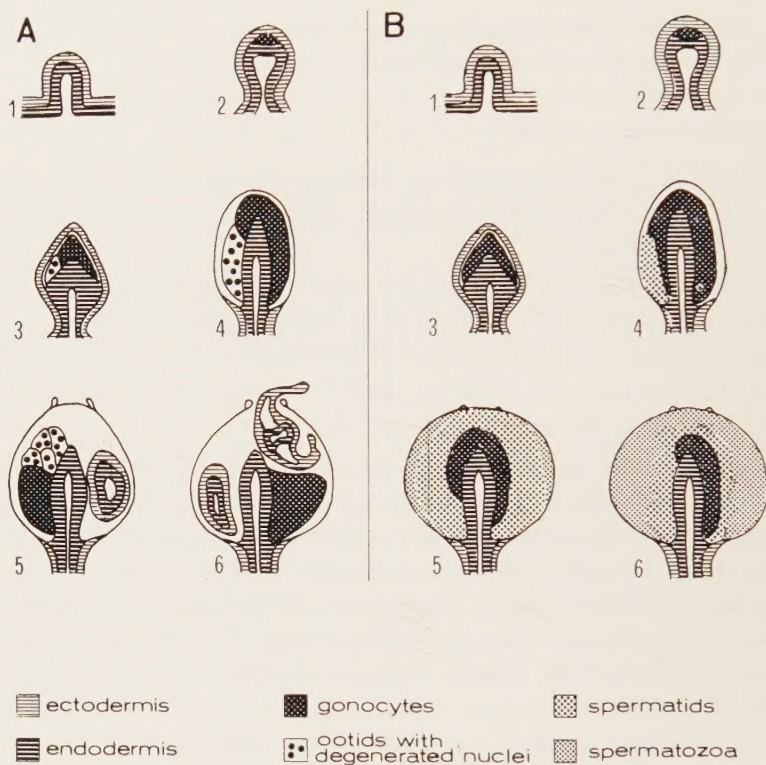


FIG. 1. Normogenesis of the female (A) and male (B) gonophores of *Tubularia*. The formation of the outer membrane is simplified in the drawing (3-6). In fact it consists of an ectodermal and a folded, double endodermal layer. A 6: Female gonophore with hatching actinula.

1.1.3. Loss of hydranths due to predators and degeneration phenomena

In colonies living under natural conditions there is always a certain percentage of individual stems regenerating hydranths which had been previously lost. This loss may be due to the following reasons: predators, degeneration or spontaneous autotomy. As far as I could observe, the first two factors mentioned have to be considered, but play a relatively unimportant role. Spontaneous autotomy as described and analysed in detail in the following chapters is the main reason for hydranth removal.

SCHOENENBERGER (personal communication) has observed that the Opisthobranchs, *Facellina drummondi* and *F. dubia*, feed on hydranths of *Tubularia*

ingesting them whole. Furthermore I have found an as yet unidentified species of TURBELLARIA (ACOELA) which attaches itself to the hydranth by means of its pharynx. This attack results in a complete dissolution of the hydranth. Both predators, snails and Turbellarians, lay their eggs between or on the stems or stolons of the colony. Fig. 2 A shows a hatching Turbellarian.

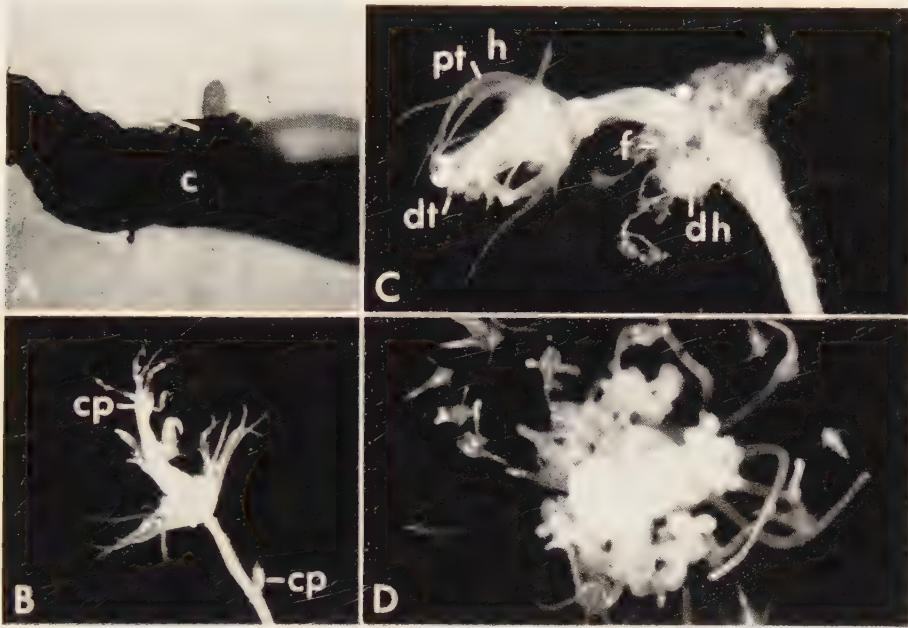


FIG. 2. A: Turbellarian (tr) hatching from the egg (e) fixed to a caulus (c) of *Tubularia*. B: A hydranth engulfing a caprellid (cp). Another caprellid sits on the stem. C: A newly formed hydranth (h) being expelled through tissue clump (dh) of a degenerated head. Fungi (f) grow on the disaggregated material. pt = proximal tentacles, dt = distal tentacles. D: A hydranth feeding on *Artemia* 2 days after autotomy.

Different species of the genus *Caprella* (AMPHIPODA) are found to invade the colonies of *Tubularia* in large numbers. These combionts are not able to injure the hydranths and therefore are not responsible for head removal, but they occasionally may be observed feeding on actinulae. Sometimes specimens of the Caprellids are ingested by *Tubularia* hydranths (Fig. 2 B). They are not immune to the nematocysts. When their ventral side or head is brought into contact with the tentacles, the animals die within one or two hours. Only the legs appear to be sufficiently armed to allow them to crawl over the hydranths.

Hydranth removal by a degeneration process has been observed on various occasions. The first sign of degeneration consists of a progressive shortening and stiffening of all tentacles. At this stage the hydranth has lost the capability

of catching prey. At a more advanced stage the tentacles are completely resorbed and the whole hydranth disintegrates to a clump of tissue which remains attached to the stem. Following disintegration of the hydranth, the underlying stem may initiate regeneration of a new head which pushes through the mass of degenerated cells (Fig. 2 C). Sometimes the tissue clump may also be lost before formation of a primordium takes place. This autolytic process has repeatedly been confused with true autotomy (cf. BERILL, 1948).

1.2. Autotomy of the hydranth

MORSE (1909) describes autotomy of the hydranth as being preceded by the formation of a constriction appearing in the stem-tissue just below the neck. After the tissue connections between hydranth and stem are disconnected, water movements carry away the hydranth which, according to MORSE, will disintegrate within few hours.

My observations show that induced and spontaneous autotomy occurs along a series of fairly defined events. Its beginning is characterized by the appearance of an incision within the coenosarc, sometimes in the form of a small ringlike constriction of the ectodermis and endodermis about half a millimeter behind the neck region. Sometimes this incision appears as a contraction of a more extended (about $\frac{1}{2}$ mm) area (Fig. 3 A). At this stage the ectoderm separates locally from the perisarc. While the caulus forms a blastema-like cap closing the coenosarc cavity, the proximal part of the hydranth shows signs of disintegration (Fig. 3 B, 3 C). This process is often accompanied by periodic longitudinal contractions of the hydranth and the neck region, thus exerting a mechanical stress on the region where separation takes place. But apart from periodical movements (cf. JOSEPHSON and MACKIE, 1965), the neck region alone may remain contracted, while the hydranth moves independently or not at all. After the complete separation of hydranth and caulus, the disintegrated tissues of the neck region, mainly endodermal material, remain in the empty space of the perisarc (Fig. 3 D). The detached hydranth is then carried away by water movements.

Fig. 4 shows longitudinal histological sections through the caulus and neck region, and represents the stages A, B, D as defined in Fig. 3. There is a concentration of interstitial cells in the wound closure of the stem. The tissue of the neck closes without agglomeration of I-cells.

1.3. Frequency of autotomy under natural conditions

Among freshly collected colonies of *Tubularia crocea* there is always a certain number of cauli which are about to regenerate missing hydranths. Replacing hydranths is therefore a phenomenon which occurs in the natural

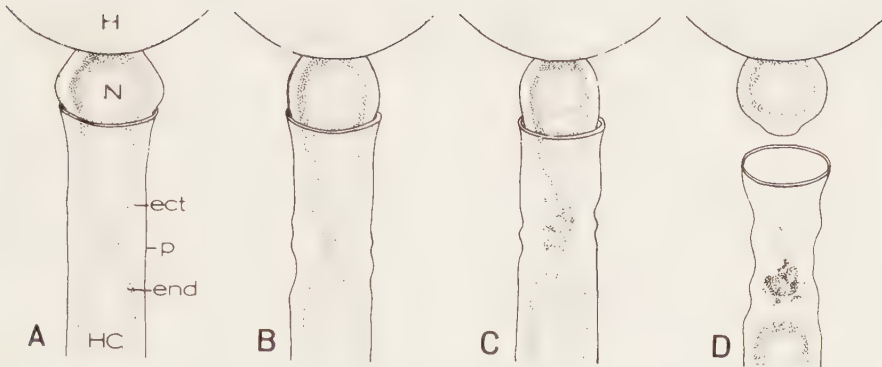


FIG. 3. A-D: Spontaneous autotomy of the hydranth (H). Sequence of events in the neck (N) and stem tissue in the hydrocaulus (HC). ect. = ectodermis, end = endodermis, p = perisarc tube.

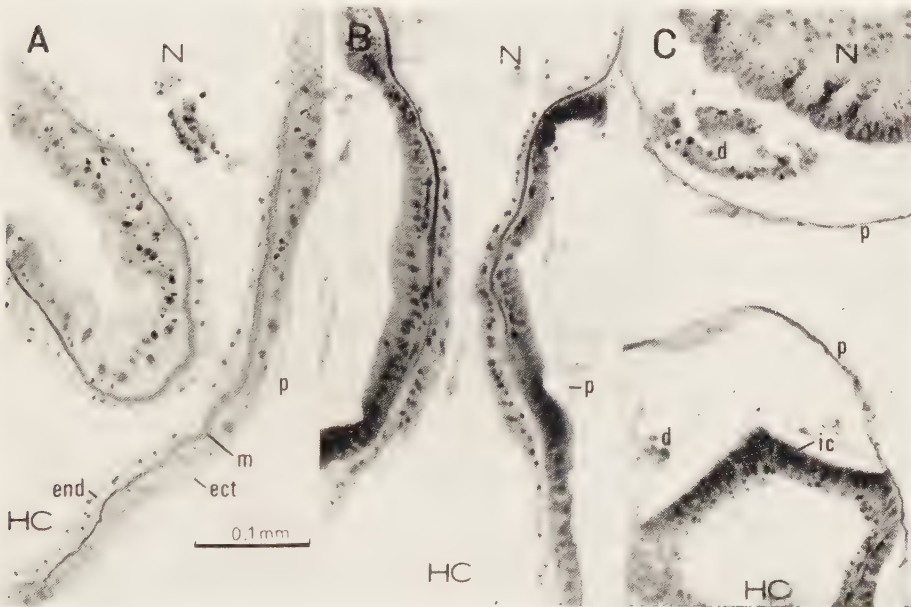


FIG. 4. A-C: Longitudinal sections through the region where the neck tissue (N) detaches from the stem tissue (HS). A = Fig. 3 A, B = Fig. 3 B, C = Fig. 3 D. (A, B, C on the same scale). p = perisarc tube, m = mesoglea, ect = ectodermis, end = endodermis, ic = interstitial cells, d = disaggregated tissue. In C the perisarc is folded and therefore cut, but does not separate (see Fig. 3 D).

environment. It was however not known whether autotomy occurs spontaneously and repeatedly in growing and maturing colonies, or whether this shedding of hydranths is the response to an ecological stress situation in which the colony enters a general regression period.

Such a regression phenomenon might be due to seasonal changes in the environmental conditions. MOORE (1939) writes: «When ocean water rises above 20°-21°C in the Woods Hole region the hydranths of *Tubularia crocea* are lost and the coenosarc retreats into the perisarc. The colonies remain in this dormant condition until the temperature drops in autumn. Growth is then resumed.» (p. 104). MACKIE (1966) believes in a similar summer regression of *T. crocea* in the Villefranche area. TARDENT (personal communication) on the other hand has observed more or less pronounced depression in Naples population during the winter season. For *T. larynx* PYEFINCH and DOWNING (1949) describe an autumnal regression which, probably due to endogenous factors, is not very regular in its sequence. None of these observations is supported by quantitative data.

During a period covering 2 years (1966-1968) I therefore determined the relative frequency of immature, mature and missing hydranths in the natural population of Santa Lucia. These data given in Fig. 5 are based on a total of 59 colonies sampled at various seasons (29 different days). The counting was done 2 to 3 hours after the colonies had been detached from their natural substratum. The stems without hydranths were separated into 2 classes, those showing an inner terminal hydranth regenerate (primordium) and those without. Short stolonial endings were not counted.

The presence of primordia indicates that the hydranth had been autotomized, when the colony was still in its natural environment. The time until the colonies were counted or fixed was too short to allow reconstitution of a hydranth autotomized during transportation. On the other hand it is impossible to say how many of the missing heads were shed in the sea and how many during transport. For the statistical evaluation, however, it was assumed that the relative rate of autotomy due to transport accidents remained constant throughout the year.

Fig. 5A summarizes the results of these countings, while Fig. 5B represents the result of one observation carried out in the sea. In Tab. 1 the averages of present hydranths, both mature and immature, in freshly collected colonies, are listed together with the statistical probability for homogeneity in different units of time. The values have been tested in χ^2 tests partitioning the degree of freedom in contingency tables (MAXWELL, 1964).

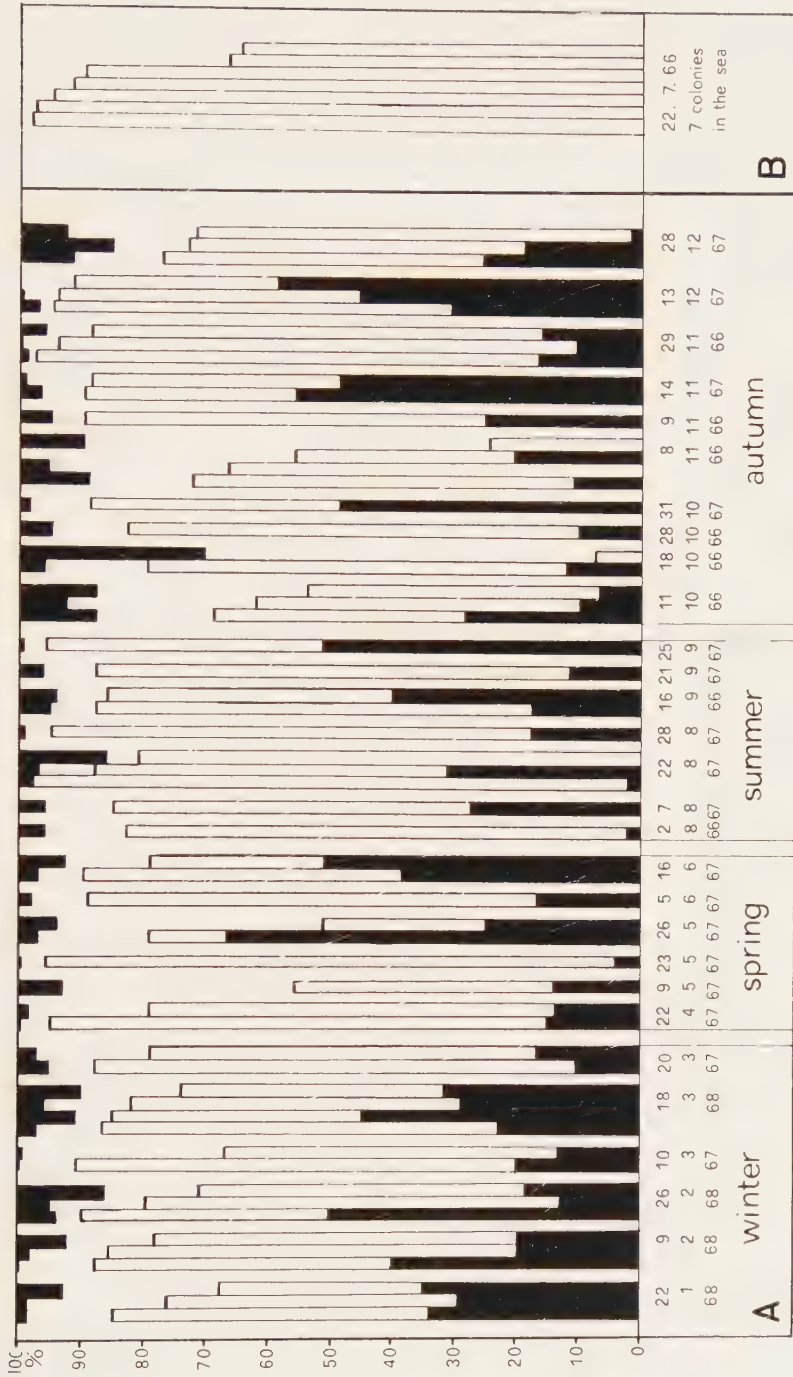


Fig. 5. A: Frequency of mature (black part of column) and immature (white part) hydranths in colonies collected throughout the year. The black columns above represent the frequency of hydranth primordia. The empty space between the basal column and the black ones on the top show the frequency of cauli bearing neither hydranths nor primordia. Each column stands for one colony. B: Frequency of present hydranths both mature and immature ones, in seven colonies counted in the sea on the same day.

The following 4 seasons have been classified according to the observed behaviour of water temperatures:

- winter 1/1 - 31/3 cold water (between 11° and 15°C)
- spring 1/4 - 30/6 warming up (from 15° to 23°C)
- summer 1/7 - 30/9 warm water (between 24° and 27°C)
- autumn 1/10 - 31/12 cooling down (from 26° to 14°C)

As shown in Tab. 1 the structure of the colonies respectively population with respect to the presence of immature and mature hydranths is highly heterogenous. There is also a considerable heterogeneity between two seasons

TAB. 1. *Structure of population.*

Time	Mean frequency of present hydranths	Total number of counted stems	Total number of counted colonies	χ^2	p for homogeneity
winter	0.797	1029	17	32.71	< 0.01
spring	0.800	625	9	183.97	< 0.0005
summer	0.886	1185	10	20.79	< 0.02
autumn	0.770	1856	23	306.28	< 0.0005
total population	0.809	4695	4 *	68.39	< 0.0005
winter-spring	smallest difference		2 *	1.37	> 0.3
summer-autumn	largest difference		2 *	51.47	< 0.0005
1 day: 18/ 3/68	0.826	161	4	3.36	> 0.4
1 day: 8/11/66	0.585	106	4	13.37	< 0.005
1 day: 22/ 6/66 sampled in the sea	0.905	723	7	132.28	< 0.0005

* = number of seasons compared.

(summer, autumn), but this does not indicate seasonal fluctuation because the same degree of heterogeneity is found between colonies from the same place collected on the same day. This is also true of colonies counted in the sea which therefore were not subjected to transport mishaps.

We can therefore say that the Naples population does not show a seasonal regression cycle. In all seasons colonies are found which are composed of immature and mature hydranths and of stems that have shed their heads. In winter, however, it is more difficult to find huge colonies, but this decrease is due to storms that tear off the colonies and to the fact that the old barrels are replaced by new, painted ones.

There are the same differences in the structure of colonies collected on the same day and those of different seasons. This observation suggests that ecological stresses do not play a predominant role in inducing autotomy. The phenomenon must be controlled by the endogenous condition of the colony itself (cf. chaps. 2.1-2.5.). As will be shown in the following chapters, however, the frequency of autotomy can also be modified by changing some factors of the physicochemical environment.

1.4. Intercolonial correlation of colony-structure

Three colonies of *Tubularia* (19, 24, 60 stems respectively at the beginning, 24, 28, 75 after the experiment) were kept in the circulating sea water of the institute in the same container. During the experiment these colonies remained

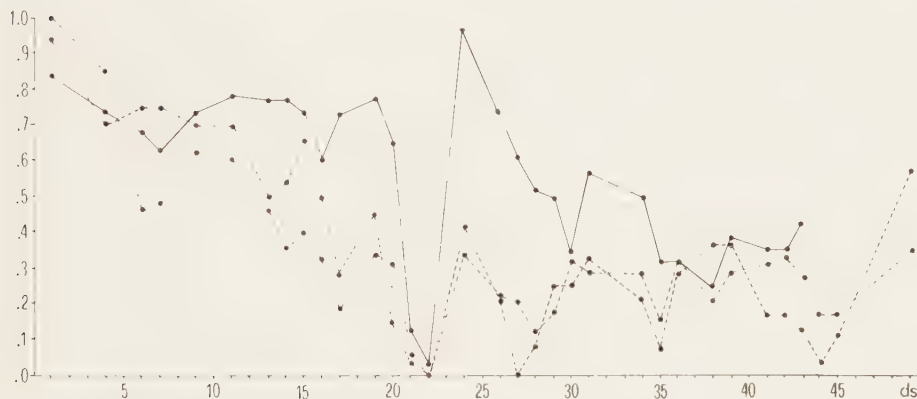


FIG. 6. Autotomy and regeneration in three colonies kept in the same tank. Each curve represents the frequency of present heads in one colony in relation to time.

attached to their natural substratum (Balanids) which could be fixed to a piece of plexiglass without damaging the stolonial system of the colonies. The temperature during the experiment oscillated between 18° and 22°C, salinity between 37.61 ‰ and 38.37 ‰, $\text{NO}_3\text{-N}$ 0.002 to 0.0045 mg/l and $\text{PO}_4\text{-P}$ 0.18 to 0.245 mg/l (the chemical analyses were performed by the staff of the institute). The water was aerated by means of a vacuum pump. The animals were fed daily on plankton and *Artemia*. Fig. 6 shows the frequencies of present hydranths as recorded during the 43 day period. The values obtained from the 3 colonies show remarkable differences between one another, but the changes in the frequencies show generally the same tendency. These changes between 2 subsequent checks do not represent the true values for autotomies or rege-

nerations which occurred, because they may compensate or overcompensate each other. Simply the difference between the two phenomena was recorded.

To test whether the apparent correlation of tendencies is statistically significant, the frequencies in each colony on the different days have been ranked according to the method for determining the Kendall coefficient of concordance (with ties) (SIEGEL, 1956). With a $W = 0.817$, $N = 30$, $k = 30$ and $df = 29$, the resulting X^2 is 71.12 and the probability for non-correlation is $p < 0.001$. This means that there is in fact a correlation with respect to the dynamics of colonies kept under identical environmental conditions. On the other hand, the daily differences in the absolute frequency of heads between the colonies proves that the general state of the colony is also involved as already suggested in the previous chapter.

General attention should be paid to the fact that the tendency to autotomize, exhibited by freshly collected colonies, favours correlation. It must be emphasized however that these colonies are not subjected to a general regression, because they continued feeding and regenerated hydranths matured. To explain the strong regression on the 20th day, it should be mentioned that on the two preceding days the circulation did not function and the water started to stagnate. For the other days there is no obvious correlation between the known water conditions and the frequencies of present hydranths. These values are therefore not enumerated in detail. In the following chapters the influence of single, artificially-modified conditions is illustrated.

1.5. Influence of temperature on autotomy

MACKIE (1966) kept *T. crocea* in Villefranche at 14°C and observed only a « few » cases of spontaneous autotomy. Accordingly he concludes that autotomy occurs at temperatures above 18°C or because of « poor water conditions ». MOORE (1939) also relates the summer regression in the Woods Hole region to high temperatures indicating 20°-21°C as the critical threshold. MORSE (1909) believes that temperature is the only factor which under natural conditions can release autotomy. While at 8°C his polyps did not autotomize for a period of 3 weeks, 33°C would induce autotomy within 2 hours. He also noted that the frequency of autotomy was higher at 25°C compared with 20°C. BERILL (1948) compared these facts with the conditions existing at Woods Hole, but as already mentioned, his data refer not only to true autotomy but also to autolysis of the hydranths (cf. chap. 1.2.).

The experiment which was planned to investigate the influence of temperature on the frequency of autotomy was carried out in 2 parallel series. Each series consisted of three groups of 15 animals carrying mature gonophores. The groups were kept at constant temperature of 13°, 16° and 21°C

respectively. The animals were fed and the water was changed daily and the number of remaining, not autotomized heads recorded.

Fig. 7 gives the results of this experiments. It shows that autotomy in isolated polyps occurs more rapidly at higher temperatures.

By using the method of BEHRENS and KÄRBER (v.d. WAERDEN, 1957) the average time until half of the animals shed their was calculated. As the results of the 2 series are in accordance, the values were computed for both together ($N = 30$). The average time until autotomy occurred was 9.0 ± 0.3 days at 13°C , 4.4 ± 0.2 days at 16°C and 2.1 ± 0.1 days at 21°C . These periods do not have to be considered as the average length of presence of a hydranth, which would mean the time between emergence of a regenerate and autotomy, but may be used to compare the influence of changed temperature on samples grown in another temperature. This comparison shows that there are ecological stresses, as, for example, temperature, which can delay or promote autotomy.

The true presence of hydranths on the stem after regeneration until autotomy was recorded under laboratory conditions in isolated stems used for growth registration (cf. chap. 2.1.). It is about 18 days at 15°C and about 14 days at 18°C . In Fig. 8 the average times hydranths remain attached to the stems are drawn in relation to temperature (MORSE, 1909 and own results).

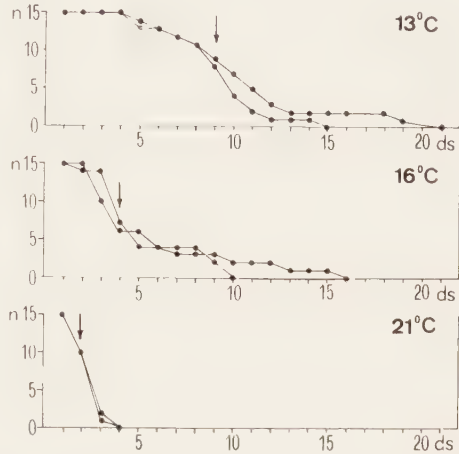


FIG. 7. Influence of temperature on autotomy. Two independent series of 15 isolated animals were observed in each temperature. The number of present, non-autotomized heads is given in relation to time (days).

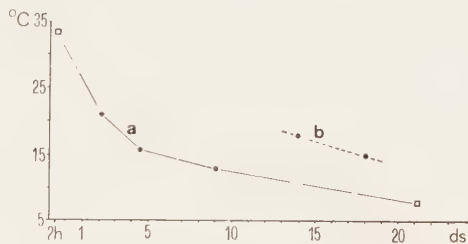


FIG. 8. Relation between temperature and the average time heads remain attached to the stem. a: From the presence-time in the temperature series (Fig. 7) a mean value was compiled and is given in relation to temperature. The rectangles represent values given by MORSE (1909). All values in this curve were received from animals taken from the sea and exposed to the new temperature given in the experiment. b: Mean presence-time of heads (regeneration until autotomy) in specimens grown under constant temperature in the laboratory. These times are also given in relation to temperature.

1.6. Influence of oxygen and carbon dioxide

MORSE (1909) found that autotomy occurs in all animals within 2 hours when they are transferred to boiled water. On the other hand high O_2 -concentrations do not enhance the resistance to autotomy. According to BARTH (1937) the rate of regeneration in *Tubularia* increases when the PO_2 is raised. LOOMIS (1958) induced gametogenesis in *Hydra* by exposing the animals to relatively high $P CO_2$. BRAVERMANN (1962, 1963) obtained the same result with *Podocoryne carnea*.

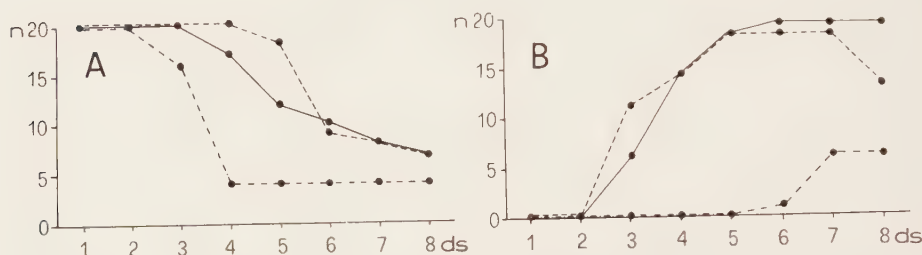


FIG. 9. Influence of increased O_2 and CO_2 -pressures on autotomy (A) and regeneration (B). The number of non-autotomized, respectively regenerated hydranths is given in relation to time.

— = control series under normal conditions

--- = raised O_2 -pressure ($P = 1$)

- . - . = raised CO_2 -pressure ($P = 0.02$)

The technique by which the gas pressure was modified (100 % O_2 , 2 % CO_2 respectively) is described in «material and methods». The animals were isolated from freshly collected colonies and distributed as follows:

— 20 cauli with	hydranths in oxygen	series (1)
— 20 » without	» » »	» (2)
— 20 » with	» » carbon dioxide	» (3)
— 20 » without	» » » »	» (4)
— 20 » with	» » normal atmosphere	» (5)
— 20 » without	» » » »	» (6)

(The hydranths in series 2, 4 and 6 were amputated)

For a period of 8 days following the beginning of the experiment the number of non-autotomized hydranths (series 1, 3, 5) or the number of regenerated hydranths (series 2, 4, 6) was recorded. The results are given in Fig. 9.

The experiments show that spontaneous autotomy (Fig. 9A) occurs more rapidly under high $P CO_2$ (3) than in the controls (5). An increased O_2 -concentration does not exclude autotomy, but slightly delays its start (1), compared with the controls (5). Conforming to this effect, regeneration (Fig. 9B) is

completely inhibited under increased CO_2 -concentration for four days and starts only in a few animals afterwards (4), while stems in the oxygen box regenerate earlier (2) than the controls (6) (cf. BARTH 1937). Both normal polyps and regenerating cauli under high P CO_2 show an interesting particularity. Four animals which had not autotomized their hydranth within 4 days after the beginning of the experiment kept them as long as the experiment lasted (8 days) (3). Although the P CO_2 remained constant throughout the experiment, regeneration (4) was not completely suppressed but delayed (4 days). Both observations indicate that there is a physiological adaptation to high CO_2 -pressures.

1.7. Behaviour of autotomized hydranths

Little was known about the behaviour of isolated hydranths following autotomy. They were considered to disintegrate within a few hours after separating from the colony (MORSE, 1909; BERILL, 1948). However TARDENT (1963) thinks that liberated hydranths remain alive for a considerable time thus replacing to a certain extent the missing medusa generation.

In a series of experiments I studied the behaviour of spontaneously autotomized hydranths with respect to their life expectancy, behaviour and sexual activity.

A total of 25 plankton samples were collected at various distances from the normal habitat of *Tubularia crocea*. The net, the opening of which was 1 m in diameter, was horizontally dragged along the surface over a distance of 50 m, or vertically from 10 to 0 m depth. Only 2 immature hydranths were found together with 2 actinulae in one of the samples taken in the immediate vicinity of the barrels. I therefore cannot give any information about the distances covered by actinulae and autotomized hydranths. The presence of two intact hydranths however shows that autotomy does occur in the natural environment, whereby healthy (neither decaying nor injured) heads are liberated.

According to observations made by PYEFINCH and DOWNING (1949), the sinking speed of actinulae larvae is 1 mm/s. Immature hydranths of *T. crocea* sink at a speed of about 1.5 mm/s, mature ones sink at about 3 mm/s. These measurements were made in a glass cylinder. The speed was recorded between 15 and 28 cm depth. Twenty-four of the 28 hydranths observed spread their tentacles while sinking in non-agitated water. This also occurred when they were dropped into the cylinder with a smaller pipette and therefore were completely closed at the beginning. Only 2 contracted again while sinking. In currents produced in aquaria they often float with the proximal tentacles rigidly folded backwards, the peristome hanging downwards.

Hydranths autotomized during transport and amputated ones were kept

floating in aerated off-shore water. The standard dishes were cleaned daily. Under these conditions the heads could be kept alive for up to 30 days after autotomy. The hydranths fed on *Artemia* nauplii while floating as well as while lying on the ground (Fig. 2D). They are also able to regenerate lost parts (see p. 128). Sooner or later the hydranths die by autolysis in the same way as described for degenerating heads on the stem (see p. 101).

In order to investigate whether spontaneously autotomized hydranths continue to produce eggs and spermatozoa and whether fertilization is still possible, different series of isolated hydranths were kept under conditions as described above.

- In series A 8 mature males, 4 females and 2 immature hydranths were kept in the same container.
- In series B 4 mature males and 4 immature females were kept together in order to observe whether fertilization is possible after autotomy.
- In series C 12 mature females were kept without males to determine the duration of spawning when no continuous fertilization was possible.
- In series D finally, 2 immature females served as a control to test whether the stage considered to be immature really did not yet contain fertilized germs.

Fig. 10 shows the rate of survival of the representatives of these 4 experimental series (uninterrupted line). The same illustration indicates the total rate at which spawning occurred (dotted lines) in the hydranths of the respective series.

The high spawning activity on the first days (series A and C) can be due to the fact that germs without tentacles were prematurely expelled from the gonophores together with complete actinulae. Accordingly the spawning rate of the 3rd and 4th day after autotomy was considerably lower. On the eleventh day the production of actinulae stopped. While the female hydranths which had reached maturity before autotomy and which were kept without males (series C) did not resume spawning after the 11th day, the females in series A (together with mature males) produced actinulae on the 12th day again. The females which were immature when autotomy occurred, and which were kept together with mature males (series B) started spawning after the 11th day. It is not clear why in series A there is this break on the 11th day. However as all series suggest that the lapse between fertilization and spawning is about 11 days, it might be an effect of unknown external or endogenous conditions during autotomy which prohibited fertilization. The isolated females of series D and also of other controls, using amputated heads, never produced actinulae, which proves that in the other series (A and C) fertilization took place after autotomy.

As observed in another experimental series, hydranths amputated 1 day

after regeneration do not reach maturity, even when kept together with mature males. Two days after regeneration, heads may autotomize or be amputated and subsequently reach maturity within a minimum of 8 days. In the time needed to mature there is no obvious difference between isolated hydranths

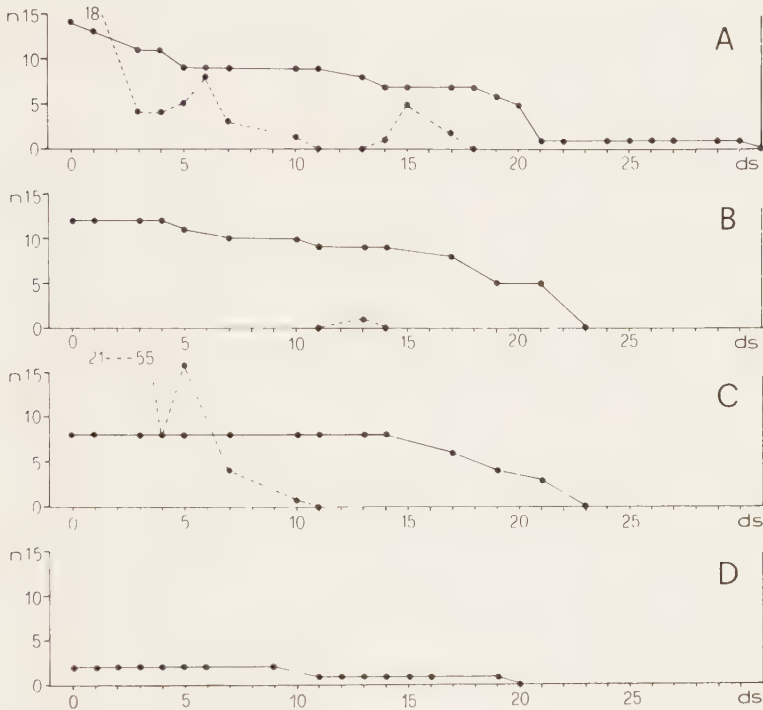


FIG. 10. A-D: The number of surviving autotomized hydranths is drawn in relation to days after autotomy (full lines). The number of actinulae spawned per day by all hydranths together is given by dots connected by interrupted lines. A: 8 males, 4 females, 2 immature hydranths at the beginning (series A cf. text). B: 4 males, 4 immature females (series B). C: 12 females (series C). D: 2 immature females (series D).

and those on the cauli that mature 8 to 14 days after emerging from the perisarc, if they do not autotomize before.

These experiments on the behaviour and life expectancy of isolated hydranths can be summarized as follows: Spontaneously autotomized hydranths survive isolation from the colonies for a considerable period of time. While floating in the water they continue to feed. They are also capable of regenerating missing parts. In hydranths which have reached sexual maturity before autotomy occurs gametogenesis continues after isolation from the colony, and isolated hydranths that were immature when shedding took place, can mature.

All these observations suggest that autotomy of hydranths is not only a

response to changing environmental conditions as, for instance, high temperatures or increased $P\text{CO}_2$, but also a cyclic phenomenon. From an ecological point of view the liberated hydranth is to a certain extent compensating for the missing medusa stage because it can contribute to the horizontal distribution and propagation of sexual products.

PHYSIOLOGICAL ASPECTS OF AUTOTOMY

2.1. *Growth*

TARDENT'S (1965) experiments on regeneration in which he used isolated cauli of various lengths suggested that longitudinal growth is possible only in connection with and after regeneration. According to his observations, subterminal growth of the hydrocaulus is particularly pronounced after the reconstituted hydranth has emerged from the perisarc tube. This statement is not in accordance with the findings of MACKIE (1966) who recorded growth of 1 normal, non-regenerating specimen over a period of 17 days as well as 5 specimens over «shorter» periods. In these specimens which belonged to a young colony kept under conditions in which autotomy occurred only rarely, MACKIE found a more or less constant growth rate of about 1 mm/d. But he adds: «When they were finally measured at the end of the study, however, they were shorter than they should have been. The longest, then 42 days old, was only 35 mm long.» (p. 403).

As the question whether growth is in a causal relationship to regeneration or not is still the object of controversy I studied longitudinal growth of hydrocauli under various conditions.

Series 1 (15°C): In order to measure longitudinal growth in isolated polyps during all stages of the autotomy-regeneration cycle, 21 animals (caulus and hydranth) were separated from freshly collected female and male colonies. The length of these cauli, the proximal ends of which were ligatured by means of nylon-loops, ranged between 10 and 30 mm. In order to observe the influence of regeneration on growth, the hydranths were amputated and, after reconstitution of the head had taken place, the lengths of the cauli were recorded in 24-hour intervals for a period of 14 days. The animals were fed daily. During this period 8 specimens spontaneously autotomized their regenerated hydranths. Autotomy was then followed by a second regeneration. Among the other 13 polyps, 6 males and 1 female reached maturity within 9 to 14 days after post-operational regeneration of the hydranth.

Series 2 (18°C): This series served the same purpose as series 1, but the animals were kept at 18°C, in order to check to which extent temperature influences the process of growth. The 21 polyps were prepared and kept in

the same way as described for series 1. Here a total of 10 specimens performed a second regeneration following spontaneous autotomy of the hydranth. From the remaining 11 specimens 6 males matured within 9 to 14 days, while the others remained immature.

Series 3 (21°C): In this series the rate of growth was recorded not in isolated specimens as in the preceding series but in intact colonies reared from single polyps which were brought in from the sea. These polyps formed a stolonial system which was attached to the glass surface of the dish. After a few days the tips of the stolons detached from the substratum and produced secondary polyps.

Three such colonies, each of which consisted of 1 primary, 2 secondary polyps and 2 to 3 stolonial endings, were kept in standard dishes at 21°C. They were fed on *Artemia* and measured daily over a period of 12 days. During this time spontaneous autotomy occurred 9 times (in 2 stems twice).

The behaviour of the animals of all 3 series as to their autotomy regeneration cycle can be subdivided into the following phases:

- Regeneration-phase: Formation of a hydranth-primordium and differentiation of the regenerate (TARDENT, 1963).
- Post-reconstititional phase: Includes the 3 days following emergence of the reconstituted hydranth.
- Intermediate phase: Covers the period from the 4th day after regeneration up to 3 days before autotomy takes place.
- Pre-autotomial phase: Corresponds to the 2 days preceding spontaneous autotomy of the hydranth. During this time the incision already described in chap. 1.2. is being formed.
- Autotomy-phase: This phase includes the complete separation of the neck and stem tissue and the removal of the hydranth.

The measured growth rates refer to these phases. The results obtained were statistically analysed with Student's t-tests. All results are given in Tab. 2 A and their statistical comparison in Tab. 2 B. The average growth rates measured in the different series during the respective phases are represented in Fig. 11. For practical reasons, the following description of the results starts with the intermediate phase which serves as a basic value to which all the others are referred.

Intermediate phase

Series 1: The 13 isolated stems which did not perform autotomy during the observation-period (14 days) gave an average growth rate of 1.56 ± 0.11 mm/d. The recorded growth rate of the polyps which shed their heads during the experimental period was 1.63 ± 0.24 mm/d during the corresponding phase.

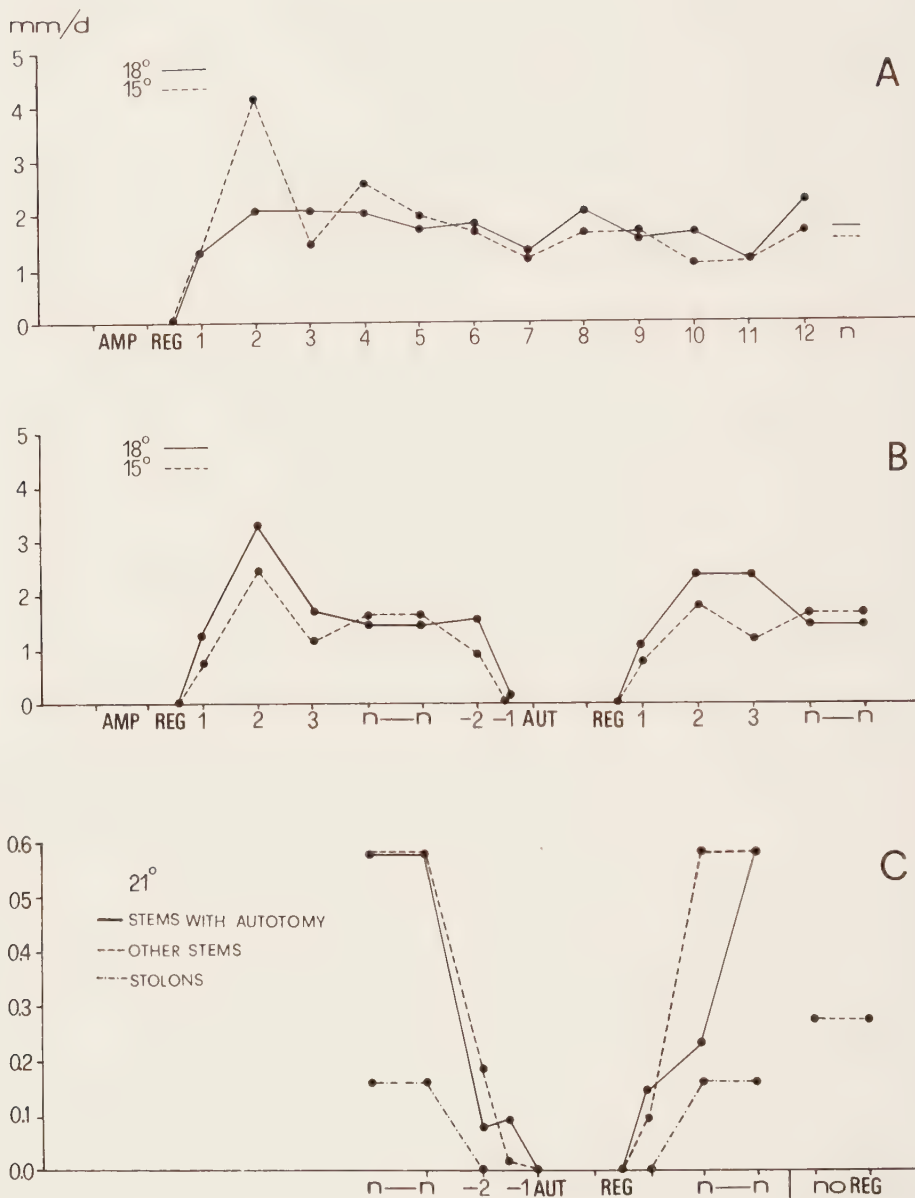


FIG. 11. Average growth rates (mm/d) in different periods of the life cycle (see p. 115). A: single stems not autotomizing during the experiment. B: single stems autotomizing during the experiment. C: colonies. AMP = amputation, AUT = autotomy, REG = regeneration. 1, 2... 12 = days after reconstitution. -2, -1 = days before autotomy, n = average intermediate growth (days 4 to n cf. text), no REG = average growth rate in a colony, while another member of the colony did not regenerate (2 days).

Series 2: The 11 non-autotomizing specimens grew at an average speed of 1.68 ± 0.16 mm/d, while the autotomizing animals grew 1.44 ± 0.23 mm/d during their intermediate phase.

As to growth of isolated stems within the intermediate phase there is no significant difference either between the 2 temperature series 1 and 2, or between the autotomizing and non-autotomizing specimens of each series. I therefore used the mean value 1.60 ± 0.07 mm/d for further statistical comparison.

Series 3: The growth rate of specimens integrated in colonies was considerably lower than that of isolated stems (series 1 and 2). The average was 0.58 ± 0.06 mm/d and stem. The demonstrated difference between the average intermediate growth of isolated stems (series 1 and 2) and colonial stems (series 3) is significant with a resulting $p < 0.01$.

Pre-autotomial phase

Series 1 and 2: Two days before spontaneous autotomy reaches its critical stage, growth is slowed down or stops completely. It measures 1.29 ± 0.23 mm/d two days, and 0.15 ± 0.02 mm/d one day before shedding of the hydranth occurs. The difference between the average growth rates of the intermediate and the preautotomial phase (0.73 ± 0.14) is significant ($p < 0.01$).

There is a complication to be considered: When the animals were checked and a hydranth was found to have autotomized, autotomy had actually occurred anything between 24 and 0 hours before the check. Thus, the animal was allowed to grow for an average of only 12 hours before growth came to a standstill. But nevertheless the difference recorded between the intermediate phase and the pre-autotomial phase is significant even if, for the explained reason, the measured growth rate of the last day before autotomy is doubled ($p < 0.01$).

Series 3: In colonial individuals, entering the pre-autotomial phase, growth is also slowed down to 0.08 ± 0.04 mm/d on the second and 0.10 ± 0.06 mm/d on the last day before autotomy. It is interesting to note that in the non-autotomizing individuals of the same colony growth comes also to a near standstill (0.19 ± 0.11 mm/d, 0.02 ± 0.02 mm/d respectively). The medium growth of both pre-autotomial days in autotomizing and non-autotomizing specimens is significantly different from intermediate growth ($p < 0.01$). This is also true if the rates of the second day are doubled for the reasons mentioned above. In this phase also the stolons stopped growth completely.

Autotomy-phase and regeneration-phase

Series 1 and 2: While the hydranth is autotomizing and while the primordium of a new hydranth is formed, there is no longitudinal growth whatsoever.

Growth is resumed only after the reconstituted hydranth has emerged from the perisarc tube.

Series 3: As in series 1 and 2, colonial individuals autotomizing and regenerating do not show any longitudinal growth.

When autotomy occurred in a specimen which was an integrated part of a colony, regeneration was sometimes not completed within the usual 24 to 40 hours. In this case I recorded the growth rates of the other individuals of the same colony for 2 days. In this particular case the growth rate was slightly lower (0.36 ± 0.025 mm/d) than the average growth rate which is typical for the normal intermediate phase but this difference is statistically not significant (few cases).

Post-restitutional phase

Series 1 and 2: Two days after the regenerated hydranth has emerged from the perisarc, subterminal growth of the caulus is resumed and accelerated (2.8 ± 0.26 mm/d) compared with the growth rate of the intermediate phase ($p < 0.01$). The highest value recorded in one specimen was 8.5 mm/d.

What has been said about the day before autotomy (p. 117) is also valid for the first day of the post-restitutional phase: When in a daily check a regenerated hydranth was found it could not be said at which time, within the preceding 24 hours, it had emerged and for how long therefore growth had already taken place. We therefore assume an average time of 12 hours. This circumstance may be responsible for the seemingly slow resumption of growth immediately after reconstitution of the hydranth (1.17 ± 0.09 mm/d).

On the third day after reconstitution growth (1.68 ± 0.17 mm/d) is within the range of intermediate growth.

Series 3: Contrary to what has been observed in isolated polyps, colonial cauli do not show an increased rate of growth during the postrestitutional phase. The average value of 0.18 ± 0.036 is even lower than that for the intermediate phase, but this difference is not significant.

From these observations we can conclude that the autotomy-regeneration phenomenon affects growth, but the growth pattern of isolated individuals is at the same time different from that of polyps which are part of a colony. In both cases the initiation of autotomy leads to a cessation of growth, which is complete during autotomy itself and is not resumed before the regenerated hydranth has emerged from the perisarc tube. Post-restitutional growth is much faster in isolated cauli than in colonial individuals, where growth is resumed at a slower pace. It is interesting to note that, when the autotomizing animal is attached to the colony, not only growth of this particular specimen is affected but also that of all individuals, which are in the intermediate phase, of the same colony. This observation suggests that a common growth-regulating

TAB. 2 A. *Growth rates.*

Sample	n	$\bar{x} \pm m^{**}$	
		mm/d	mm/d
INTERMEDIATE PHASE			
<i>Single stems</i>			
a) 15°C non-autotomizing	117	1.56	0.11
b) 15°C autotomizing	37	1.63	0.24
c) 18°C non-autotomizing	99	1.68	0.16
d) 18°C autotomizing	30	1.44	0.23
e) average intermediate phase	283	1.60	0.07
<i>Colonies</i>			
f) average intermediate phase	29	0.58	0.06
PRE-AUTOTOMIAL PHASE			
<i>Single stems</i>			
g) 2 days before autotomy	21	1.29	0.23
h) 1 day » »	20	0.15	0.02
i) both days before »	41	0.73	0.14
j) » » (h doubled)	41	0.81 *	
<i>Colonies</i>			
k) 2 days before autotomy in own stem	10	0.08	0.034
l) 1 day » » » »	6	0.10	0.062
m) 2 days » » other »	8	0.19	0.11
n) 1 day » » » »	5	0.02	0.018
o) all stems 2 days before autotomy	18	0.13	0.05
p) » » 1 day » »	11	0.06	0.038
q) » » both days before »	29	0.10	0.037
r) » » » » (p doubled)	29	0.13 *	
POST-RECONSTITUTIONAL PHASE			
<i>Single stems</i>			
s) 1 day after reconstitution	60	1.17	0.093
t) 2 days » »	58	2.82	0.26
u) 3 days » »	52	1.68	0.17
<i>Colonies</i>			
v) all stems 1 day after reconstitution	5	0.14	0.054
w) » » 2 days » »	4	0.23	0.035
x) both days after reconstitution	9	0.18	0.036
y) » » (w doubled)	9	0.26 *	
z) if in other stems no regeneration	5	0.36	0.025

* = explanation in the text (p. 117).

** $m = S' \sqrt{n}$.

TAB. 2 B. *t*-tests

Samples compared (cf. tab. 2 A)	n	t	Difference	
			significant p	not significant p
a and b	154	0.33		> 0.1
c » d	129	0.93		> 0.1
a + b » c + d	283	0.10		> 0.1
e » f	312	4.63	< 0.01	
e » i	324	4.48	< 0.01	
e » j	324	4.07	< 0.01	
f » q	58	4.07	< 0.01	
f » r	58	3.85	< 0.01	
e » t	368	7.48	< 0.01	
e » u	335	0.45		> 0.1
f » x (n = 9)	38	1.94		> 0.05
f » y (n = 9)	38	1.58		> 0.05
f » z (n = 5)	34	0.72		> 0.1

principle depending on the autotomy-regeneration cycle acts within the whole colony.

This growth-regulatory principle may well be related to an inhibitor substance. The existence of such an inhibitor, which controls the reconstitution of the hydranth in *Tubularia* has been demonstrated by several authors (ROSE, 1963, 1966, 1967 a + b; ROSE and POWERS, 1966; TARDENT, 1956, 1960; TARDENT and EYMANN, 1958, 1959; et. al.). Moreover BURNETT (1966) collected experimental evidence suggesting that all morphogenetic activities of *Hydra* depend on interactions between a stimulator and an inhibitor.

The possible connections between the growth pattern of *Tubularia* as described above and such an inhibitory mechanism will be discussed later.

2.2. Interacting hydranths and the influence of cuts

According to BURNETT's model (1966) the fast-diffusable inhibitor must permanently leak out of the animals surface. This loss of inhibitor is, according to BURNETT's view, particularly intense when the animal has been injured by a cut. Because of this local loss of inhibitor, the balance shifts in favour of the stimulator which induces cell proliferation and regeneration. BURNETT (personal communication) proposes a similar regulatory principle for *Tubularia* where, in contrast with *Hydra*, the inhibitor would be retained in the coenosarc because of the impermeable perisarc tube.

In order to test whether the inhibitory principle postulated in the previous chapter is based on such a fast-diffusing substance which would diffuse following cuts and the concentration of which could be increased by grafting hydranths close together, a few pilot-experiments were carried out.

Series 1: In this series 8 polyps showing incisions below the neck (stage B, Fig. 3), thus initiating autotomy, were isolated from freshly-collected colonies

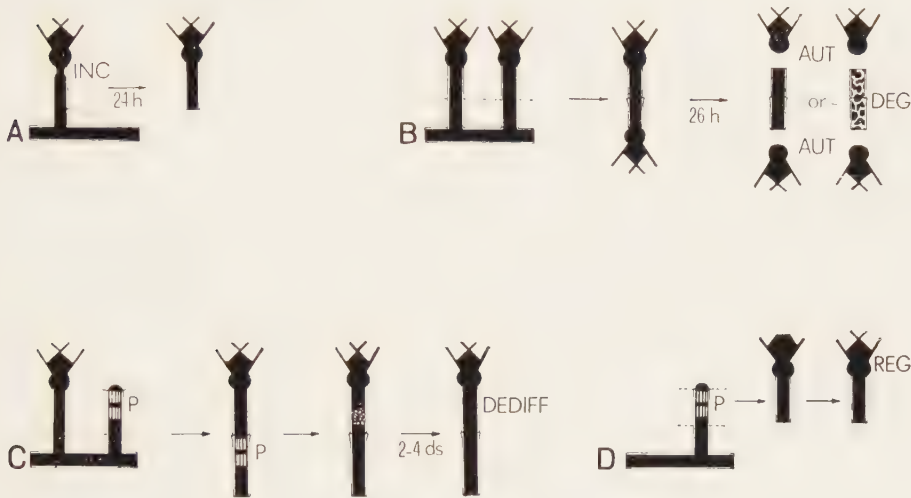


FIG. 12. A: Reconstitution of the incised neck region after cutting the caulus below the incision (INC) (series 1). B: Grafting of 2 hydranths with opposite polarity. The samples autotomize (AUT) whereby the stem may disintegrate (DEG) (series 2). C: Grafting of primordia with same polarity behind a hydranth. The dedifferentiating tentacle ridges and the pigmented area migrate distally until they disappear completely (DEDIFF) (series 3). D: Control to C, Regeneration in primordia the most distal regions of which were cut as necessary for grafting.

by a cut situated 5 mm below the incision. These specimens were kept in standard dishes at 18°C. 24 hours after isolation, 7 of the 8 animals had completely reconstituted the incised neck region (Fig. 12 A). By separating the specimens from the colony it was possible to stop the process of autotomy which had started when the polyps still were attached to the colony.

As will be shown later, however, the presence of the hydranth on isolated cauli which do not show an incision at the beginning of the experiment cannot be prolonged by repeated cutting of the proximal end of the stem (p. 125).

Series 2: If the hydranth is the site of inhibitor production and the inhibitor favours autotomy, it should be possible to speed up autotomy by grafting one hydranth in the immediate neighbourhood of another. The effect of such a situation has been tested by the following experimental set-up:

Two hydranths were grafted with opposite polarity one upon the other, so that the resulting stem piece between them was 4 to 6 mm long (Fig. 12 B).

All 6 hydranths of the 3 produced couples autotomized within 26 hours. In addition, in one specimen the coenosarc tissue connecting the opposite hydranths showed signs of disintegration.

Series 3: By opposing hydranths primordia of different developmental stages TARDENT and EYMAN (1959) demonstrated that the more advanced a primordium is the stronger its inhibitory action on younger primordia. In order to test whether hydranth primordia produce a sufficient amount of inhibitor for releasing autotomy in hydranths situated in their neighbourhood, the following grafting combination was made:

Four primordia in which both the distal and proximal tentacle ridges were differentiated were grafted with the same polarity behind a caulus bearing an intact hydranth (Fig. 12 C). These pieces were kept in standard dishes at 18°C.

The grafted primordia did not continue to differentiate but dedifferentiated within 2 to 4 days following operation. Dedifferentiation was accompanied by a distal migration of the pigmented area and the gradually disappearing tentacle ridges (Fig. 12 C). It is not clearly understood how this distal migration of the primordium occurred.

In this experiment, the grafted primordium did not induce autotomy of the hydranth above it. On the contrary, the hydranth, probably by its inhibitor production brought about a dedifferentiation of the already well-advanced regenerate. Such dedifferentiation was obtained by TARDENT and EYMAN (1959) when advanced primordia were exposed to high concentrations of extracts obtained from adult hydranths.

That dedifferentiation is actually due to the presence of the hydranth and not to the removal of a small distal portion of the primordium, as necessary for the grafting operation, is shown by the following control.

The distal caps of normal primordia at the same stage as used for grafting, were removed (Fig. 12 D). In spite of this partial mutilation the primordia completed their differentiation and produced a normal hydranth as already shown by DAVIDSON and BERILL (1948). Moreover we have seen (Fig. 2 C) that behind a completely degenerated hydranth formation of a primordium is possible.

However, the question whether dedifferentiation of primordia behind a grafted hydranth is due to inhibition by the hydranth or to lack of oxygen is not clear. As differentiation of an already formed primordium is not possible behind a hydranth, formation of a primordium does not seem to be a reason for autotomy.

Conclusively it can be said that the grafting experiments have given further evidence for a causal relationship between the hydranth-produced inhibitor and the phenomenon of autotomy. The fact that autotomy, once initiated, is reversible if the caulus is opened in the vicinity of the hydranth

(series 1) suggests that the accumulated inhibitor favouring autotomy was at least partly withdrawn by diffusion from the cut surface, it must be said, however, that the reversibility of autotomy is restricted to the first stages and is possible only as long as the dissociation of the neck tissue has not started (i.e. until stage B, Fig. 3).

When 2 inhibitor-producing hydranths are placed in close contact by grafting (series 2), thus accumulating the inhibitor within a small, closed system, the process of autotomy is released immediately.

The confrontation of fully-differentiated hydranths and their primordia (series 3) indicates that the amount of inhibitor produced by the latter is not sufficient for inducing autotomy. It is the hydranth which seems to cause dedifferentiation of the primordium.

As these grafting experiments have provided evidence for an inhibitor-induced autotomy, it was checked whether autotomy can be released and enhanced by substance(s) obtained by diffusion and by extraction. This problem is the object of the following chapter.

2.3. Influence of extracts on autotomy

The growth pattern of autotomizing polyps (cf. chap. 2.2.) suggest that one of the factors controlling autotomy is an inhibitor which may be identical with that involved in regeneration.

In fact, active extracts inhibiting hydranth reconstitution in *Tubularia*, were obtained by simple diffusion (ROSE and ROSE, 1941) and by homogenization of hydranths (TARDENT and EYMANN, 1958, 1959; TWEDELL, 1958 a + b, 1962). More information about regional specificity and the behaviour of the regeneration inhibitor is given by ROSE (1963, 1966, 1967 a + b), ROSE and POWERS (1966) and AKIN and AKIN (1962).

FULTON (1959) attributed the inhibitory effect of diffusion water on regeneration to metabolites produced by bacterial growth in such solutions. Precaution has therefore been taken in the following experiments to avoid bacterial growth or, in any case to compare the influence of diffusion waters with other extracts (homogenate) that might be favourable to bacterial growth.

Besides the inhibitor acting as a morphogenetic regulator, there is at least in *Hydra* also an antagonistic stimulator. The existence of such a factor which is probably of neurosecretory origin and is heat stable, has been demonstrated by LESH and BURNETT (1964, 1966) and LENTZ (1965). A comparable stimulator has not yet been found to act in *Tubularia*.

In the following experimental series the aim of which was to check the influence of diffusion waters and of various extracts on the process of autotomy I used preparatory techniques as described by TARDENT and EYMANN (1958), LESH and BURNETT (1964, 1966) and LENTZ (1965).

Series 1: Diffusion water from about 500 freshly collected polyps was prepared (diffusion time 30 minutes, total volume 300 ml). The solution was filtered through paper.

30 polyps of various length bearing intact hydranths were transferred to a standard dish containing diffusion water. 30 other polyps serving as controls were kept in sea water under identical conditions (18°C). The behaviour of the

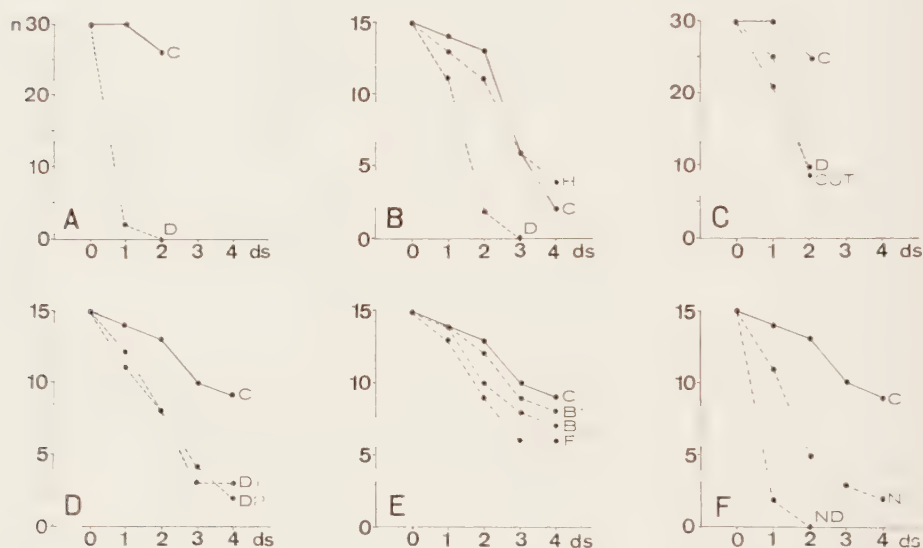


FIG. 13. Influence of different extracts on autotomy. Non-autotomized polyps in relation to time. A: D = diffusion water, C = control (series 1). B: D = diffusion water, H = extract from homogenates, C = control (series 2). C: D = diffusion water, CUT = animals cut daily at the proximal stem ending, C = control (series 3). D: D₁ = diffusion water (series 4 a) (centr. 1000 g/15'), D₂ = diffusion water (series 4 a) (centr. 25000 g/50'). E: B₁ (series 4 a), B₂ (series 4 b) = extracts from homogenates (25000 g/50') without fast diffusable components. F = as B₁ B₂ but centr. at 2500 g/10'. F: DN = nematocyst poison and diffusion water (series 4 b), N = nematocyst poison without diffusion water (4 a), C = control animals, same in whole series 4.

experimental animals and of the controls as to the frequency of autotomy was observed.

Already one day after the start of the experiment all except 2 polyps of the experimentals had autotomized their hydranth. In the remaining 2 specimens autotomy occurred during the following day. Of the control animals none had autotomized during the first day. Two days after beginning of the experiment only 4 out of 30 had lost their hydranths. Fig. 13 A gives the frequency of autotomy of the experimental and control animals in relation to time. The result clearly shows that autotomy is accelerated by diffusion water (D).

Series 2: 500 fractionated polyps were left to stand for 2 hours in 300 ml sea water. After that they were filtered out of this diffusion water and were

roughly homogenized in a glass tube. The homogenate was filtered (paper) and the extract was diluted with pure sea water up to a final volume of 300 ml. The solid material was discarded. The procedure thus gave 2 extracts to be tested: 1. the initial diffusion water (D) and 2. the extract which was obtained by homogenizing the solid fraction after a diffusion time of 2 hours (H).

45 polyps of various length were isolated from freshly-collected colonies and split into 3 groups.

15 specimen were transferred to the diffusion water D, 15 to the solution H and 15 to pure sea water (controls C). Each group was kept in a standard dish containing 300 ml of solution at 15°C.

The result of this series is given in Fig. 13 B, which shows that all animals which were exposed to diffusion water D had autotomized their hydranth within 3 days. In the 2 other groups, solution H and the controls C, the hydranths were shed at a slower rate. There is no clear difference between the behaviour of the specimens of groups H and C.

In this series again diffusion water was the most active in releasing autotomy, while the extract obtained from the material after a diffusion time was not active compared with the control. This implies also that metabolites produced by bacterial growth play no part, or only an unimportant one, in enhancing autotomy.

Series 3: In this series diffusion water was prepared by a 4 hour diffusion and then filtered through a 3G5 filter immediately before it was used. 30 isolated polyps were, for 18 hours, transferred to this solution. After that they were transferred to pure sea water for 6 hours, fed, then brought again into freshly-filtered solution for 18 hours (18°C).

The result of this experiment is shown in Fig. 13 C. The curve D indicates that, compared with the controls C, the polyps exposed to the diffusion water autotomize at a higher frequency. This effect can again be attributed to the inhibitor(s) present in the solution D.

It was shown in chapter 2.2. that the initiated process of autotomy can be reversed if the diffusable inhibitor is permitted to leak out of the system through an open cut surface. By promoting this loss of inhibitor by means of repeated traumatization of the proximal end of the caulus it should be possible to decrease the frequency of spontaneous autotomy. In order to check this hypothesis I isolated 30 polyps from freshly-collected colonies and amputated the proximal ends of their stems daily, on the first day a second time after 6 hours.

These specimens (Fig. 13 C, CUT) autotomized at the same rate as the polyps exposed to diffusion water. This result is in contradiction with the previous experiment (cf. chap. 2.2.) in which, by creating an open cut surface near an already initiated incision, reconstitution of the neck region was induced. The reason for this discrepancy is not clear.

Series 4: The diffusion waters as prepared in the previous series contain

only fast diffusing substances, according to BURNETT'S model of *Hydra* (1966) inhibitor. This series is dedicated to the problem of whether in other fractions of extracts other factors acting on autotomy can be detected. The *Tubularia* extracts from homogenates were therefore fractionated in a way similar to that in which LESH and BURNETT (1964) and LENTZ (1965) handled their material for isolating stimulator from *Hydra*.

a) About 1000 freshly collected polyps (hydranths and stems) were minced with scissors and stored for diffusion at room temperature for 90 minutes in 300 ml of sea water. After filtration through paper the diffusion water was divided into 2 portions of 150 ml each.

Fraction D₁: Portion 1 of the diffusion water was centrifuged at 1000 g for 15 minutes (TARDENT and EYMANN, 1958). The supernatant of this portion represents fraction D₁. The sediment of this portion was discarded.

Fraction D₂: Portion 2 of the diffusion water was centrifuged at 2500 g for 50 minutes. The sediment was discarded while the supernatant was used as fraction D₂.

Both fractions were stored at -20°C and thawed immediately before being used and diluted 1:9 in « instant ocean » (37 ‰).

Portions of 15 isolated polyps were transferred to the fractions D₁ and D₂ and to pure « instant ocean » (C). The behaviour of these animals with respect to autotomy is given in Fig. 13 D. It is in accordance with the result obtained with diffusion waters of the preceding series (Fig. 13 A, B, C).

Fractions N, F, B₁: The tissue mass from which the diffusion water (D₁ and D₂) was obtained was, after filtration, boiled in 100 ml of distilled water for 10 minutes. This treatment was aimed at discharging the nematocysts. The whole mass was filtered through paper. The liquid fraction supposedly containing also nematocyst poison was centrifuged at 1000 g for 15 minutes. The supernatant was used as fraction N.

The sediment obtained by centrifugation was homogenized in 20 ml of distilled water. It was divided into 2 portions of 10 ml each. The first portion was centrifuged at 2500 g for 15 minutes and the supernatant kept as fraction F.

The second portion was centrifuged at 25000 g for 50 minutes. The sediment was discarded while the supernatant was used as fraction B₁.

15 polyps each were subjected to the action of fraction N, F and B₁ to which after storage (-20°C) 9 parts of « instant ocean » (41 ‰) were added (see p. 97). The behaviour of the polyps at to autotomy was compared with 15 controls (same in whole series 4) kept in pure « instant ocean ».

The results represented in Figs. 13 E and 13 F show that fraction N (Fig. 13 F) probably containing nematocyst poison (but little diffusion water) strongly promotes autotomy. Shedding of the heads is slightly enhanced by the fractions B₁ and F but to a much smaller extent than by diffusion waters D₁ and D₂. It is possible that this weak effect exhibited by the fractions B₁ and F is due to

residual amounts of inhibitor(s) which had not completely diffused out of the tissue.

b) Fractions DN and B₂: In this experimental group extracts were made of hydranths only. About 500 hydranths were cut from the stems and boiled for 10 minutes in 100 ml of distilled water. After filtration through paper the liquid probably containing the fast-diffusing, heat stable (TARDENT and EYMANN, 1958) inhibitor as well as nematocyst poison was centrifuged at 1 000 g for 15 minutes. The sediment was discarded and the supernatant used as fraction DN.

The boiled hydranths were homogenized in 10 ml of distilled water. The homogenate was centrifuged at 25 000 g for 50 minutes (LESH and BURNETT, 1964). The sediment was discarded and the supernatant containing the same components as B₁ was used as fraction B₂.

As shown in Fig. 13 E fraction B₂ had, as expected, about the same effect on autotomy as fraction B₁ (see above). Fraction ND (Fig. 13 F) had by far the strongest effect.

The outcome of these experiments (series 1-4) permits the following conclusions. The frequency of autotomy is always greatly enhanced when the extract to which the animal is exposed contains fast-diffusible components as obtained in simple diffusion waters (fractions D). This fast-diffusing component is most probably identical with the inhibitor substance(s). Another fraction (N, ND) is obtained by boiling the minced mass, thus probably discarding the nematocysts or solubilizing residues of the diffusible substance(s). However the fractions (H, B₁, B₂, F) which contain little fast diffusing components (homogenized after a diffusion period) promote a much lower incidence of autotomy.

The fact that hydranths which have autotomized under the influence of autotomy-promoting extracts continue to live and that cauli regenerate when brought into normal sea water, indicate that the observed effects are not due to the presence of bacteria as has been claimed by FULTON (1959) in the case of the regeneration inhibitor. There is no reason to believe that the active diffusion waters are more contaminated than the extracts obtained from homogenates.

2.4. Influence of colchicine on autotomy

KANATANI found that in *Dugesia gonocephala* colchicine and demecolcine enhance the formation of bipolar heads (1958) and that transversal fission may occur (1960). WEBSTER (1967) was able to induce alteration of polarity in *Hydra* by colcemide (demecolcine).

In order to test whether colchicine affects the frequency of autotomy a series of 15 polyps with distal hydranths were kept in different concentrations of colchicine (M/3000, M/5000, M/8000) at 15°C. The animals serving as a control were kept in pure off-shore water.

Series 1: 15 polyps isolated from freshly collected colonies were kept in standard dishes containing a solution of colchicine M/5000, M/8000 respectively and in pure sea water. The solutions were changed once, i.e. 3 days after the start of the experiment, after the animals had been fed with *Artemia*. The specimens in the M/5000 colchicine concentration all autotomized their heads 5 days after the beginning of the treatment. In M/8000, of the 15 specimens 12 had autotomized within the same period, while in pure sea water only 6 of the 15 controls had shed their hydranths.

Series 2: The experimental set up of this series was the same as for series 1 but colchicine was applied in higher concentrations (M/3000, M/5000). The rate of autotomy of 15 polyps each was recorded.

All polyps exposed to M/3000 autotomized their heads within 2 days, while of the 15 specimens kept in M/5000 14 had shed their hydranths. The remaining autotomized on the 3rd day. Of the 15 control animals in pure sea water, 5 shed their heads on the 3rd day. All remaining 10 hydranths detached from the stems by the 5th day.

The results of these experiments can be summarized as follows. Colchicine does not prolong the presence of hydranths on the stem but increases the rate of autotomy. According to KANATANI (1960) the reason for this effect might be destruction of polarity in the animal.

2.5. Cell proliferation

The results of the chapters 2.1., 2.2., 2.3. indicate that autotomy is subjected to the control of a fast-diffusible inhibitor substance(s) present within the colonies. The question is whether this inhibitor is the only relevant factor or whether autotomy is favoured by other factors, too, as for example a process of senescence (TARDENT, 1962, 1963, 1965) to which the aging hydranth could be subjected. Senescence would mean in this case a progressive exhaustion of cellular resources which could not be replaced by a continuous supply of cells from the caulus i.e. from the colony. TARDENT thinks that «rejuvenation» of the terminal hydranths is possible only by means of a complete replacement of the organ. If this is true, we would expect that in an aging hydranth mitotic activity would gradually come to a standstill. As a consequence of this the morphogenetic activities would also cease. TARDENT has not checked this hypothesis experimentally.

This chapter describes experiments concerned with the reconstititional abilities and the rate of mitotic activity in hydranths of various stages.

2.5.1. Regeneration potential of hydranths

The hydranths to be tested were cut transversally just above the level where the gonophores originate, in order to observe whether proximal fragments

are able to regenerate the missing distal parts, namely distal tentacles and hypostome. The proximal fragments including the blastostyles, proximal tentacles and the basal part of the hydranth were kept in agitated off-shore water in Bovery dishes at 18°C. A total of 26 hydranths were tested. Regeneration was recorded as positive when the hydranth fragment had reconstituted the distal tentacles and the hypostome, and was able to feed. It was negative when these structures were not formed and only wound closure occurred.

Series 1: 11 hydranths that were still attached to the stems were operated as described above. All samples, both mature and immature, showed complete regeneration of the missing distal parts within 2 days.

Series 2: 15 autotomized hydranths were operated in the same way, zero to 5 days after having detached from the caulus. A total of 7 mature and immature heads reconstituted their missing parts within 2 days.

This shows that both sessile and autotomized hydranths are capable of regenerating distal structures. This means that the latter have retained at least part of their morphogenetic abilities, a finding which is in contrast with the senescence-theory.

2.5.2. Mitotic activities in hydranths

The observation that mature and immature hydranths are able to regenerate amputated parts suggests that the cells of this organ have retained their ability to proliferate. In order to check whether the rate of mitotic activity is subjected to changes, I recorded the rate of 3H-thymidine incorporation in hydranths at various stages of their life-cycle. The injected hydranths were prepared for autoradiographic examination (see p. 97).

The hydranths were cut longitudinally and 6 consecutive sections near the middle axis were evaluated with respect to the frequency of labelled nuclei. Only one side of the body and only the ectodermis were counted. A cell was considered as being labelled when its nuclear area was covered by 10 or more silver grains.

The frequencies of labelled cells in one mature hydranth attached to the stem, 2 hydranths in incision (cf. Fig. 3, stage B, C), 2 spontaneously autotomized hydranths labelled 0 to 24 hours after autotomy, and 2 labelled from the 4th to the 5th day after autotomy were compared. The results are given in Fig. 14. They show that mitotic activity in spontaneously autotomized hydranths, even 5 days after they have detached from the colony, is about the same as that in the sessile hydranth. This indicates that autotomy is not a consequence of senescence (TARDENT, 1962, 1963, 1965).

The 2 cases representing the hydranth in its incision-stage show a lower rate. As the overall X^2 of the values given in Fig. 14 indicates heterogeneity ($p < 0.0005$), this temporary decrease of mitotic activity is significant. This phe-

nomenon is in agreement with the temporary stop of growth during this pre-autotomial phase (p. 117) and the interpretation of phase-specific inhibitor concentration (see p. 127).

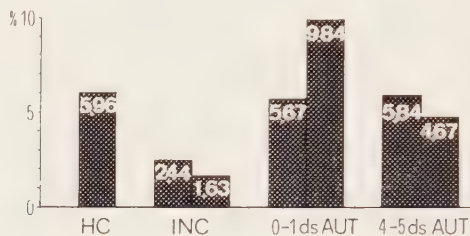


FIG. 14. Frequency of labelled cells (%) in different stages of the life cycle. For each head the average percentage taken from regions 1-6 of the ectodermis only is represented in one column. HC = hydranth on stem, INC = incision, 0-1ds and 4-5 ds AUT = days after autotomy.

In order to obtain a picture about the regional frequency of labelled cells the longitudinal sections were subdivided into the following axial regions (cf. Fig. 15).

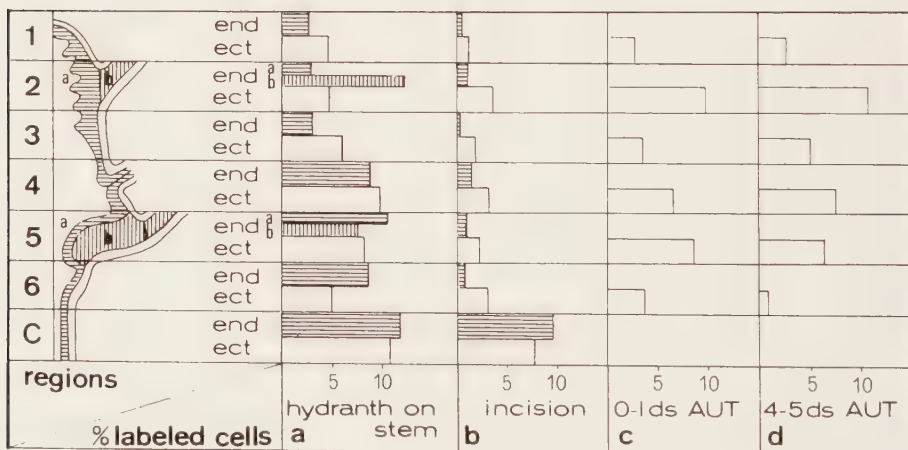


FIG. 15. Longitudinal distribution of labelled cells. The horizontal columns give the frequency (%) in the respective regions drawn on the left side (cf. text). 2 a, 5 a = gastrodermis. 2 b, 5 b = cells in the mesoglea and tentacle-endodermis. In samples c + d only the ectodermis was counted.

- 1) Hypostome
- 2) Base of distal tentacles
- 3) Cone between distal tentacles and blastostyles
- 4) Gonophore region

- 5) Base of proximal tentacles and bottom of the hydranth
- 6) Neck region

Endodermis and ectodermis of 6 consecutive slides near the middle axis were counted (Fig. 15, samples a and b). In samples c and d of Fig. 15 the frequency of labelled endodermal cells could not be determined because these cells contained, for an unknown reason, large amounts of dark inclusions which could not be readily distinguished from the silver grains indicating labelled material.

The results and a schematic drawing of the described body regions are given in Fig. 15. The labelled nuclei, representing cells that had undergone mitosis within 24 hours, are not homogeneously distributed along the longitudinal axis of the hydranth. I found labelled cells accumulated near the nerve rings, which are situated at the bases of the tentacles (BURNETT, personal communication), but also in the blastostyles and gonophore buds. Further information about the radial distribution of labelled cells is given by CAMPBELL (1967).

2.5.3. Cell migration

The labelled cells found in the hydranths may either originate from local cell proliferation or, in the case of sessile hydranths, cell migration may contribute to the cell supply. TARDENT's suggestion (1966) that senesced hydranths are replaced was based on the fact that he found no histological evidence for cell migration between caulus and hydranth, nor neoformation of e.g. cnidoblasts in the hydranth.

In order to check again whether there is an exchange of cells between the caulus and hydranth, in both proximo-distal and disto-proximal directions, an autoradiographical technique was used. Non-labelled hydranths with a short stem piece (1-3 mm), necessary for grafting, were grafted upon cauli labelled during the preceding 24 hours and isolated from the same colony. Labelled hydranths and unlabelled cauli were combined as well. Tissue continuity was established after 10 to 20 minutes. The animals were fed on the second day after grafting, and fixed after four days for autoradiographic examination (see p. 97).

In each animal 3 consecutive, longitudinal sections from near the middle axis were evaluated with respect to the average absolute number of labelled cells found in 1 mm length of stem tissue, in the different regions of the hydranths respectively. The average number of labelled cells per section and per described region in the donor tissue (x) and in the former unlabelled host tissue (y) may serve as a measure of cell migration. These numbers are given in Tab. 3.

These results indicate that migration takes place from the caulus in the direction of the hydranth. The number of cells immigrated in the neck region is

TAB. 3. *Cell migration.*

Sample	Average number of labelled cells/region and section in							
	labelled donor tissue = x				unlabelled host tissue = y			
Proximo-distal migration	caulus above graft:	caulus above graft:			hydranth region *:			
	0-1 mm	0-1	1-2	2-3 mm	6	5	4	3-1
A ectodermis	14.3	16.7			12.7	2.0	1.0	0.0
endodermis	12.3	6.7			8.3	2.0	0.0	0.0
B ectodermis	15.3	6.0	0.7	0.0	0.0	0.0	0.0	0.0
endodermis	24.3	15.0	9.0	0.2	0.0	0.0	0.0	0.0
Disto-proximal migration	caulus above graft:	caulus below graft:						
	0-1 mm	0-1	1-2 mm					
C ectodermis	25.7	0.3	0.0					
endodermis	18.7	1.0	0.0					
D ectodermis	1.3	0.0	0.0					
endodermis	0.7		0.0					

* = cf. Fig. 15.

considerable, but in the other regions it is very small compared with the number of cells undergoing mitosis in the respective regions within 24 hours (cf. Fig. 15). The speed of migration, about 3 mm within 4 days, is also slow compared with the speed of, for example, regeneration phenomena. The number of cells migrating in proximal direction is negligible. It is interesting to note that the animal D (cf. Tab. 3) which was in incision during the labelling period and reconstituted the incised neck region only after the grafting operation, shows very low mitotic activity, conforming to the observations given in Fig. 14.

DISCUSSION

Different authors have observed and commented on the phenomenon of hydranth autotomy in *Tubularia* (MORSE, 1909; MOORE, 1939; BERILL, 1948; PYEFINCH and DOWNING, 1949; TARDENT, 1952-1965; MACKIE, 1966). The significance of the phenomenon, however, still is a matter of controversy.

Autotomy is chiefly considered as being the response of the polyp or colony to changing environmental conditions (MOORE, 1939; BERILL, 1948; PYEFINCH and DOWNING, 1949; MACKIE, 1966), leading to a regression period, during which the colony remains in a dormant state. It might be assumed, that the naked part directly exposed to the external influences is shed to allow protection of the whole.

My own observations about the frequency of autotomy in the sea have shown instead that it is not directly related to certain occasional or seasonal stresses, but is found all the year round in apparently normal colonies showing immature and mature hydranths as well as primordia in regenerating cauli.

On the other hand, the correlation in different colonies under identical water conditions and the observed modifications in the frequency of autotomy at changing temperatures and raised CO₂-pressure suggest that there is an influence of ecological stresses which may even result in an epidemic shedding of all heads within a colony. However within ranges of ecological stresses that normally occur in the natural environment and which do not release epidemic autotomy, at least not in the Naples population, there are always differences between single individuals and between different colonies with respect to the frequency of autotomy. This indicates that ecological stresses are not responsible alone for releasing autotomy.

Furthermore, as has been shown, predators (TURBELLARIA and Opisthobranchs) are not a major cause for autotomy, although they feed on hydranths.

It is thus evident that endogenous factors are involved in inducing and controlling the autotomy-regeneration cycle, which appears to be a normal event of the life history of *Tubularia* colonies. This is in accordance with the opinion of TARDENT (1962) who came to the same conclusion after he had observed that the field organisation of the future hydranth is well determined in the subterminal region before the original hydranth has detached from the stem.

TARDENT (1962, 1963) suggests that this natural «rejuvenation-cycle» is due to a process of senescence to which the hydranth is subjected because of the absence of a proper cell supply. Autotomy and reconstitution would, according to his view, be the consequence of this senescence.

According to my observation cell proliferation does not cease in mature, fully - grown hydranths as well as in spontaneously autotomized heads. This indicates that senescence cannot be the initial cause of autotomy. The fact that autotomized hydranths are able to live for several weeks (see p. 112) continuing gametogenesis is also in contrast with the senescence theory.

On the other hand I can neither agree with MACKIE (1966) who rejects TARDENT's view of autotomy as being part of the life cycle, stating that «healthy» hydranths kept under favourable water conditions may live indefinitely and are not subjected to autotomy at all. Autotomy is, according to this author, the

result of accidental phenomena. Since MACKIE studied relatively young colonies which were not older than 40 days (counted from the settlement of the larva) and since he also observed a «few» cases of autotomy, his observations cannot be generalized.

We have seen that autotomy occurs also in feeding, growing and maturing animals and that autotomized animals continue gametogenesis, show cell proliferation and are even able to regenerate lost parts. They certainly cannot therefore be considered to be «unhealthy», an expression MACKIE uses but does not define. At this point I emphasize that «unhealthy» hydranths e.g. autotolysing and decaying heads may remain attached to the stem and do not autotomize in the typical manner.

Autotomy may for the above-mentioned reasons be considered as being an integrated part of the life cycle of *Tubularia*, serving various functions which will be discussed in the following paragraphs.

Spontaneously autotomized or amputated hydranths do not degenerate immediately after detaching from the caulus. They continue to ingest food and to live for up to 30 days after isolation. They also maintain their sexual activity. Eggs in the female gonophores are fertilized, fully developed larvae hatch and male gonophores continue to produce spermatozoa. Other elements of the behaviour of isolated hydranths have already been mentioned. All these accomplishments support TARDENT's view according to which the liberated hydranth of *Tubularia* compensates at least partly for the absence of a free swimming sexual generation (medusa) in this species. The liberated hydranth of *Tubularia* is thus comparable with the hydranth of *Margelopsis haeckely* which is never sessile (cf. WERNER, 1955). When female hydranths of *Tubularia* that are fixed to the colony liberate actinulae the latter tend to attach to the nearest substratum, often to the colony itself or in its immediate neighbourhood. Occasionally larvae are carried away by water movements and can be found in the plankton (see p. 111). From an ecological point of view the lack of a medusa generation certainly restricts the possibilities for a horizontal propagation of the population and the autotomized, erratic hydranths compensate for this handicap.

It is not certain whether mature hydranths once they have passed their sexual period and have exhausted the supply of gonocytes are capable of producing a new generation of gonophores. AVSET (1967/68) believes that the whole stock of germ cells is present when gonophore differentiation starts. I myself have never observed a reconstitution of sexually exhausted gonophores. In this particular sense we may speak of a «senescence» of the hydranth. If the latter remained attached to the colony, that particular individual would be unable to contribute further to sexual reproduction. Therefore autotomy and reconstitution of a hydranth not only serve the above-mentioned functions

but also prevent a situation in which the number of sterile individuals would accumulate within the colony.

The interdependence between the autotomy-regeneration cycle and caulus growth as proposed by TARDENT (1965) is partly true for isolated polyps, in so far as there is really faster growth registered in the post-autotomial phase, but not for specimens which are part of a colony. The reason for this difference may reside in the fact that, in isolated stems, most of a growth-inhibiting substance has diffused out of the system while in colonies nearby members would provide the amount of inhibitor suppressing an excessive longitudinal growth.

That the morphogenetic activity of a colonial polyp is not independent of that of other members is shown by the fact that autotomy occurring in one individual affects growth within the whole colony, at least if it is small (p.). This observation supports the view according to which autotomy and growth are subjected to a common morphogenetic factor.

My experiments indicate that this factor is identical with the regeneration inhibitor(s), the presence of which has been demonstrated by various authors (BARTH, 1938; ROSE and ROSE, 1941; ROSE, 1963, 1966, 1967 a,b; ROSE and POWERS, 1966; TARDENT, 1956, 1960, 1963; TARDENT and EYMANN, 1958, 1959; TWEEDELL, 1958 a + b, 1962; AKIN and AKIN, 1967, et al.).

The inhibitor principle is also part of BURNETT'S (1966) model of growth and differentiation in *Hydra* based on studies carried out by BURNETT (1959, 1961), BURNETT and DIEHL (1964 a,b,c), LESH and BURNETT (1964, 1966) et al.. For this model BURNETT postulates an antagonistic interaction between a fast-diffusible inhibitor and a stimulator (see also LENTZ, 1965).

According to ROSE (1963, 1966, 1967 a), ROSE and POWERS (1966) and to TARDENT and EYMANN (1958) the site of inhibitor production is the hydranth. In grafts in which 2 hydranths were opposed (p. 121) we have in fact found an acceleration of autotomy. Such an acceleration can also be induced by exposing polyps to diffusion water prepared from colonies. Diffusion water contains, according to ROSE and ROSE (1941) the regeneration inhibitor. We may therefore assume that the factor acting upon autotomy is identical with the regeneration inhibitor. This assumption is supported by the fact that an incision once initiated can be stopped and reversed if the inhibitor is allowed to leak out of the system (p. 121). This is in agreement with BURNETT (1966) who states that the inhibitor is a fast-diffusing substance.

As to the mode of action of the inhibitor we are inclined to think that one of its targets is cell division because, during the formation of the incision preceding autotomy, that is to say the stage in which inhibitor has accumulated, the number of mitotic divisions is significantly lowered. This also agrees with BURNETT'S (1966) view on the mode of action of the inhibitor.

The promoting action of colchicine on the incidence of autotomy might

be explained by a destruction of polarity in the animal (KANATANI, 1960) which would also mean destruction of normal transmission of controlling substances (ROSE, 1967 b).

Assuming that the inhibitor induces all processes in the neck region of *Tubularia* which take place when a hydranth is shed, we must ask why it affects such a restricted area as represented by the disintegrating part of the neck region (Figs. 4 and 5). Considering the fact that ROSE (1963, 1966, 1967 a), ROSE and POWERS (1966) have demonstrated a region specificity of the inhibitor action in the regenerate, we might assume that the neck region as a reacting system is particularly sensitive to the inhibitor which causes a local disintegration of the proximal neck tissue.

As to the stimulator which is an important part of BURNETT's *Hydra*-model (1966) it should be said that such a stimulator has so far not been detected in *Tubularia*. If such a stimulator is present in this species my experiments have not provided any evidence that it is directly involved in the control of autotomy. There was no effect on autotomy found in extracts that, according to the experiments of LESH and BURNETT (1964) should contain the stimulatory granules. Moreover regeneration was as fast in autotomized as in sessile hydranths (p. 129) which should not be the case if the stimulator were present in different concentrations. Finally, if the missing stimulator is a cause of autotomy, degenerating hydranths should autotomize which is also not the case. The decaying heads may remain attached to the stem until nothing but a tissue clump is left (Fig. 2 C).

The present study therefore implies that an endogenous, fast-diffusible inhibitor is produced by the hydranths and accumulated within the colony. Together with modifying ecological stresses, it is responsible for inducing autotomy. The autotomy-regeneration phenomenon is part of the normal life cycle of *Tubularia* having advantages in the sexual reproduction.

ACKNOWLEDGEMENT

I should like to express my grateful thanks to Prof. P. TARDENT who suggested the topic of this thesis, for his help in its elaboration, and also to Prof. A. BURNETT for the most helpful discussions we had. My thanks also go to the « Stazione Zoologica di Napoli » and to its staff. I am deeply indebted to the « Schweizerische Kommission für die Zoologische Station Neapel » and to the « Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung » (grant No. 3991) for their kind support. My gratitude also goes to Miss E. BRAENDLE for her help in making the drawings and to Mrs. J. KOCHER for reading the manuscript.

LITERATURE CITED

- AKIN, G. C., and J. R. AKIN, 1967: The effect of tripsin on regeneration inhibitors in *Tubularia*. Biol. Bull. **133**, 82-89.
- AVSET, K., 1967/68: On the gonophore development in *Tubularia indivisa* L. Nytt Mag. Zool. **15**, 112-117.
- BARTH, L. G., 1937: Oxygen as a controlling factor in the regeneration of *Tubularia*. Biol. Bull. **73**, 381.
- , 1938: Quantitative studies of the factors governing the rate of regeneration in *Tubularia mesembryanthenum*. Biol. Bull. **74**, 155-177.
- BERILL, N. J., 1948: Temperature and size in the reorganisation of *Tubularia*. J. exp. Zool. **107**, 455-464.
- , 1949: The polymorphic transformations of *Obelia*. Quart. J. Microscop. Sci. **90**, 235-264.
- BRAUER, A., 1891: Ueber die Entstehung der Geschlechtsprodukte und die Entwicklung von *Tubularia mesembryanthenum* ALLM. Z. wiss. Zool. **52**, 551-579.
- BRAVERMANN, M. H., 1962: *Podocoryne carnea* culture methods and carbondioxyde induced sexuality. Exp. Cell Res. **27**, 301-306.
- , 1963: Studies on hydroid differentiation. II. Colony growth and the initiation of sexuality. J. Embryol. exp. Morph. **11**, 239-254.
- BURNETT, A. L., 1959: Histophysiology of growth in *Hydra*. J. exp. Zool. **140**, 281-342.
- , 1961: The maintenance of form in *Hydra*. In: 20th growth symposium. Regeneration. New York: Ronald press, 27-52.
- , 1966: A model of growth and cell differentiation in *Hydra*. Am. Nat. **100**, 165-190.
- BURNETT, A. L., and N. A. DIEHL, 1964 a: The nervous system of *Hydra*. I. Types, distribution an origin of nerve elements. J. exp. Zool. **157**, 217-226.
- BURNETT, A. L., and N. A. DIEHL, 1964 b: The nervous system of *Hydra*. II. Control of growth and regeneration by neurosecretory cells. J. exp. Zool. **157**, 227-236.
- BURNETT, A. L., and N. A. DIEHL, 1964 c: The nervous system of *Hydra*. III. The initiation of sexuality with special reference to the nervous system. J. exp. Zool. **157**, 237-250.
- CAMPBELL, R. D., 1965: Cell proliferation in *Hydra*: An autoradiographic approach. Science **148**, 1231-1232.
- , 1967: Cell proliferation and morphological patterns in the hydroids *Tubularia* and *Hydractinia*. J. Embryol. exp. Morph. **17**, 607-616.
- CROWELL, S., 1953: The regression-replacement cycle of hydranths of *Obelia* and *Campanularia*. Phys. Zool. **26**, 319-327.
- DAVIDSON, M. E., and N. J. BERILL, 1948: Regeneration of primordia and developing hydranths of *Tubularia*. J. exp. Zool. **107**, 465-477.
- FULTON, C., 1959: Re-examination of an inhibitor of regeneration in *Tubularia*. Biol. Bull. **116**, 232-308.
- HAWES, F. B., 1958: Preliminary observations on the settlement of the actinula larva of *Tubularia larynx* (ELLIS and SOLANDER). Ann. and Mag. N. Hist. **13**, 147-155.
- JOSEPHSON, R. K., and G. O. MACKIE, 1965: Multiple pacemakers and the behaviour of the hydroid *Tubularia*. J. exp. Zool. **43**, 293-332.
- KANATANI, H., 1958: Formation of bipolar heads induced by demecolcine in the planarian, *Dugesia gonocephala*. J. Fac. Sci. Univ. Tokyo **8**, 253-270.
- , 1960: Studies on fission in the planarian, *Dugesia gonocephala*. J. Fac. Sci. Univ. Tokyo **9**, 59-66.

- LENTZ, T. L., 1965: *Hydra*: Induction of supernumerary heads by isolated neuro-secretory granules. *Science* **150**, 633-635.
- LESH, G. E., and A. L. BURNETT, 1964: Some biological and biochemical properties of the polarizing factor in *Hydra*. *Nature* **204**, 492-493.
- LESH, G. E., and A. L. BURNETT, 1966: A analysis of the chemical control of polarized form in *Hydra*. *J. exp. Zool.* **163**, 55-78.
- LOOMIS, W. F., 1958: Feedback control of growth and differentiation by carbon dioxide tension and related metabolic variables. In: 17th growth symposium. *Cell, Organism and Milieu*. New York: Ronald press, 253-294.
- LOWE, E., 1926: The embryology of *Tubularia larynx*. *Quart. J. Microscop. Sci.* **70**, 599-627.
- MACKIE, G. O., 1966: Growth of the hydroid *Tubularia* in culture. In: *The cnidaria and their evolution*. Symp. Zool. Soc. London **16**, 397-412.
- MAXWELL, A. E., 1964: *Analyzing qualitative data*, 2nd ed. London: Methuen Co. Ltd.
- MOORE, J. A., 1939: The role of temperature in hydranth formation in *Tubularia*. *Biol. Bull.* **76**, 104-107.
- MORSE, M., 1909: The autotomy of the hydranth of *Tubularia*. *Biol. Bull.* **16**, 172-182.
- NATHANSON, D. L., 1955: The relationship of regenerative ability to the regression of hydranths of *Campanularia*. *Biol. Bull.* **109**, 350.
- PYEFINCH, K. A., and F. S. DOWNING, 1949: Notes on the general biology of *Tubularia larynx* ELLIS and SOLANDER. *J. Mar. Biol. Ass. U.K.* **28**, 21-43.
- ROSE, S. M., 1963: Polarized control of regional structure in *Tubularia crocea*. *Develop. Biol.* **7**, 488-501.
- , 1966: Polarized inhibitory control of regional differentiation during regeneration in *Tubularia*. II. Separation of active materials by electrophoresis. *Growth* **30**, 429-447.
- , 1967 a: Polarized inhibitory control... III. The effect of grafts across sea water-agar bridges in electric fields. *Growth* **31**, 149-164.
- , 1967 b: The aging of the system for the transmission of information controlling differentiation. *J. Geront.* **22**, 28-41.
- ROSE, S. M., and F. C. ROSE, 1941: The role of a cut surface in *Tubularia* regeneration. *Phys. Zool.* **14**, 328-343.
- ROSE, S. M., and J. POWERS, 1966: Polarized inhibitory control... I. The effect of extracts from distal and proximal regions. *Growth* **30**, 429-447.
- SIEGEL, S., 1956: *Nonparametric statistics for the behavioural sciences*, 1st ed. New York: Mc Graw-Hill series.
- STREHLER, B. L., 1961: Aging in Coelenterates. In: *The biology of Hydra*. Coral Gables, Florida: Univ. of Miami Press 373-398.
- TARDENT, P., 1952: Ueber Anordnung und Eigenschaften der interstitiellen Zellen bei *Hydra* und *Tubularia*. *Rev. suisse zool.* **59**, 247-253.
- , 1954: Axiale Verteilungs-Gradienten den interstitiellen Zellen bei *Hydra* und *Tubulaira* und ihre Bedeutung für die Regeneration. *Wilhelm Roux' Archiv* **146**, 593-649.
- , 1956: Pfropf-Experimente zur Untersuchung des regenerations-hemmenden Stoffes von *Tubularia*. *Rev. suisse zool.* **63**, 229-236.
- , 1960: Principles governing the process of regeneration in hydroids. In: 18th growth symposium. New York: Ronald Press 21-43.
- , 1962: Morphogenetic phenomena in the hydrocaulus of *Tubularia*. *Pubbl. Staz. Zool. Napoli* **33**, 50-63.
- , 1963: Regeneration in the HYDROZOA. *Biol. Rev.* **38**, 293-333.

- TARDENT, P., 1964: Developmental aspects of regeneration in coelenterates. In: Regeneration in animals and related problems. NATO's symposium. Amsterdam: North-Holland Publ. Co. 71-88.
- , 1965: Ecological aspects of the morphodynamics of some HYDROZOA. *Am. Zool.* **5**, 525-529.
- TARDENT, P., and H. EYMANN, 1958: Some chemical and physical properties of the regeneration inhibitor of *Tubularia*. *Acta Embryol. Morphol. Exper.* **1**, 280-287.
- TARDENT, P., und H. EYMANN, 1959: Experimentelle Untersuchungen über den regenerationshemmenden Faktor von *Tubularia*. *Wilhelm Roux' Archiv* **151**, 1-37.
- TWEEDELL, K. S., 1958 a: Inhibitors of regeneration in *Tubularia*. *Biol. Bull.* **114**, 255-269.
- , 1958 b: A bacteria free inhibitor of regeneration in *Tubularia*. *Biol. Bull.* **115**, 369.
- , 1962: Inhibition of regeneration in *Tubularia* by tissue extract injection. *Biol. Bull.* **123**, 513-514.
- VAN DER WAERDEN, B. L., 1957: *Mathematische Statistik*, I. Aufl. Berlin-Heidelberg-New York: Springer Verlag.
- VOSS-BRINCKMANN, A., 1969: ANTHOMEDUSAE/ATHECATAE (HYDROZOA, COELENTERATA) of the Mediterranean. Part. I. CAPITATA. In: *Fauna e Flora del Golfo di Napoli*, Monografia **39** (in press).
- WEBSTER, G., 1967: Studies on pattern regulation in *Hydra*. IV. The effect of colcemide and puromycin in polarity and regulation. *J. Embryol. exp. Morph.* **18**, 181-197.
- WERNER, B., 1956: On the development and reproduction of the anthomedusan *Margelopsis haeckeli*. *Ann. N. Y. Acad. Sci.* **62**, 1-30.

Dr. D. RUNGGER, Stazione Zoologica di Napoli, 80121 Naples, Italy.

CURRICULUM VITAE

DURI RUNGGER, GEBOREN 25.3.1941 IN CHUR, GR. ICH BIN SCHWEIZER MIT BUE-
GERORT VERSAM, GR. NACH DER PRIMARSCHULE IN CHUR ABSOLVIERTE ICH DAS
GYMNASIUM AN DER BUENDNER KANTONSSCHULE (1954-1961), DAS ICH MIT DER MA-
TURA TYP B ABSCHLOSS.

IM HERBST 1961 BEGANN ICH MEIN ZOOLOGIESTUDIUM AN DER PHIL. FAK. II
DER UNIVERSITAET ZUERICH. ICH BESUCHTE KURSE UND VORLESUNGEN BEI DEN DO-
ZENTEN AKERT, BACHOFEN, BATSCHET, BIEGERT, BURLA, CHEN, DAUMER, HADORN,
HARTMANN-FRICK, HAUSCHTEK, HEDIGER, KUHN-SCHNYDER, LUDWIG, MARKGRAF,
NOETHIGER, PFEIFFER, REICH, ROHWEDER, RUTISHAUSER, SCHLITTLER, SCHMID,
SCHNITTER, SCHUMACHER, STUESSI, TARDENT, THOMAS, TOENDURY, TRUEMPY, UR-
SPRUNG, VAN DER WAERDEN, WAGNER, WANNER, WEISSMANN, ZIEGLER UND ZISWI-
LER.

IN DEN NEBENFAECHERN LEGTE ICH EXAMINA AB IN CHEMIE BEI PROF. VI-
SCONTINI ALS PROPAEDEUTIKUM, PALAEONTOLOGIE BEI PROF. KUHN - SCHNYDER
ALS 2. NEBENFACH UND BOTANIK BEI PROF. MARKGRAF ALS 1. NEBENFACH.

DIE SCHLUSSPRUEFUNG IN ZOOLOGIE ABSOLVIERTE ICH IM JULI 1968 BEI DEN
HERREN PROF. BURLA UND PROF. TARDENT.

DIE VORLIEGENDE DISSERTATION ENTSTAND UNTER DER LEITUNG VON PROF.
DR. P. TARDENT AN DER ZOOLOGISCHEN STATION NEAPEL, WO ICH ZUR ZEIT ALS
FORSCHUNGSASSISTENT TAEITIG BIN.

Officine Grafiche Napoletane

FRANCESCO GIANNINI & FIGLI